PAPERS AND ADDRESSES

BY

WILLIAM HENRY WELCH

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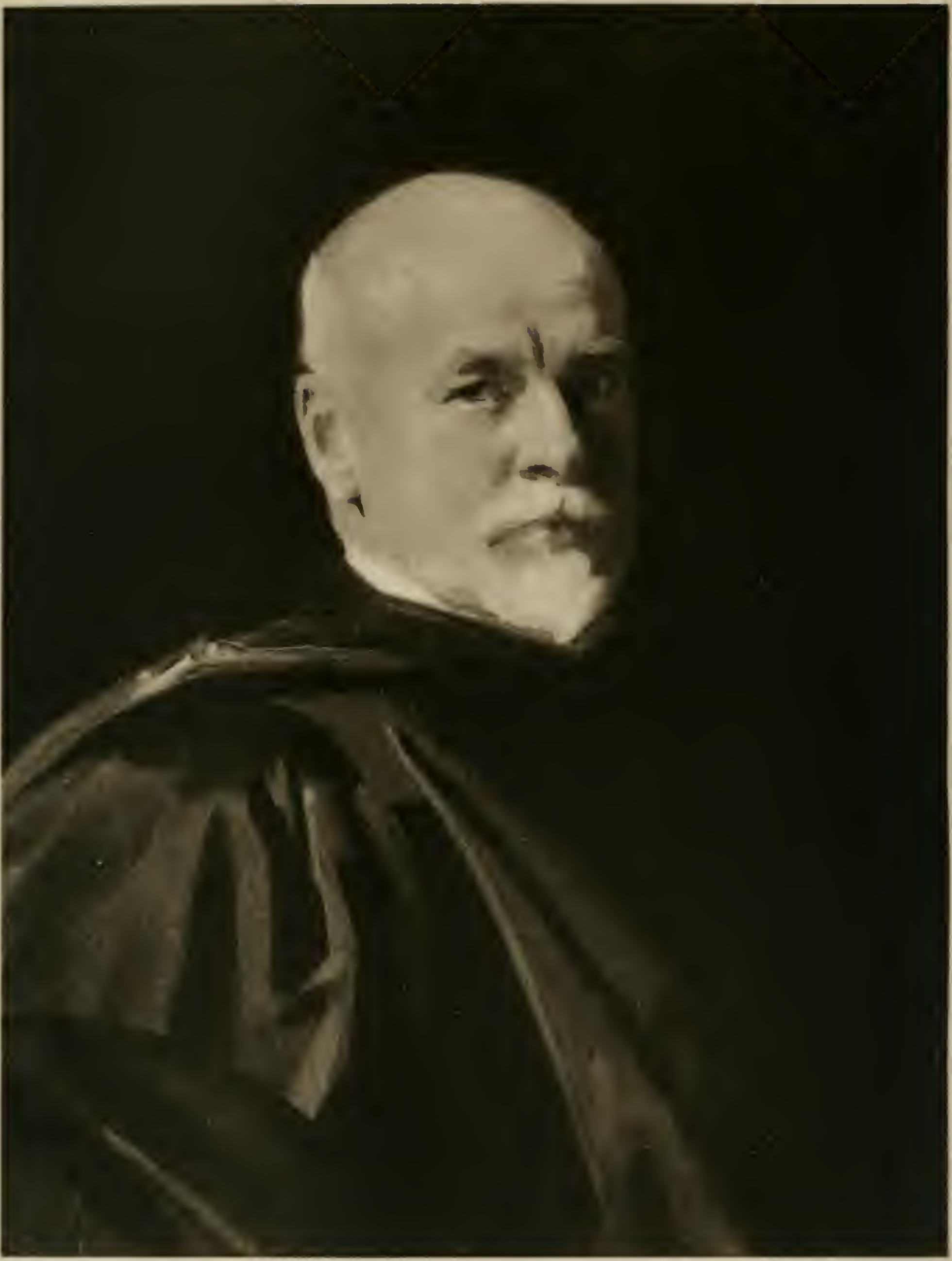

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PAPERS AND ADDRESSES

BY

WILLIAM HENRY WELCH

IN THREE VOLUMES

VOL. II

BACTERIOLOGY

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TABLE OF CONTENTS

BACTERIOLOGY

PAGE

General Considerations Concerning the Biology of Bacteria, Infection and Immunity 3
Preliminary Report of Investigations Concerning the Causation of Hog Cholera 79
Remarks on Hog Cholera and Swine Plague 86
Remarks on *Diplococcus pneumoniae* 120
The Etiology of Acute Lobar Pneumonia, Considered from a Bacteriological Point of View 124
Micrococcus lanceolatus, with Especial Reference to the Etiology of Acute Lobar Pneumonia 146
The Etiology of Diphtheria 181
The Causation of Diphtheria 197
The Histological Changes in Experimental Diphtheria 218
The Histological Lesions Produced by the Toxalbumen of Diphtheria .. 225
Bacteriological Investigations of Diphtheria in the United States 229
The Treatment of Diphtheria by Antitoxin 265
Clinical and Bacteriological Diagnosis of Diphtheria 322
Bacillus coli communis: the Conditions of its Invasion of the Human Body, and its Pathogenic Properties 328
Additional Note Concerning the Intravenous Inoculation of *Bacillus typhi abdominalis;* its Survival in the Gall-Bladder 332
Experimental Production of Typhoid-Bacillus Carriers; Bacteria in Gall-Stones 335
Principles Underlying the Serum Diagnosis of Typhoid Fever and the Methods of its Application 336
The Action of Rattlesnake Venom upon the Bactericidal Properties of the Blood 358
The Huxley Lecture on Recent Studies of Immunity, with Special Reference to Their Bearing on Pathology 359
Traumatic Cerebral Abscess, with Bacteriological Examination 389
Conditions Underlying the Infection of Wounds: Including a Discussion of Disinfection with Reference to Treatment of Wounds, of the Relation of Bacteria to Suppuration, of the Resistance of Tissues to the Multiplication of Bacteria and of the Effects of Antiseptic Agents on Wounds 392

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PAGE
Some Considerations Concerning Antiseptic Surgery.............. 419
General Bacteriology of Surgical Infections...................... 436
A Gas-Producing Bacillus (*Bacillus aerogenes capsulatus,* Nov. Spec.) Capable of Rapid Development in the Body after Death.......... 539
Observations Concerning *Bacillus aerogenes capsulatus*............. 564
The Shattuck Lecture. Morbid Conditions Caused by *Bacillus aerogenes capsulatus*.. 599
Distribution of *Bacillus aerogenes capsulatus* (*Bacillus welchii,* Migula) 637
Eye Tuberculosis and Anti-Tubercular Inoculation in the Rabbit...... 638
The Changes in the Lungs in Systemic Blastomycosis as Contrasted with Those of Tuberculosis................................ 641
Mode of Infection and of Dissemination of Tuberculosis in Man, Based on Anatomical Investigation.............................. 643
What Shall be the Mode of Procedure in Determining the Pathogenesis of Bacteria Found in Water?........................ 645

BACTERIOLOGY

GENERAL CONSIDERATIONS CONCERNING THE BIOLOGY OF BACTERIA, INFECTION AND IMMUNITY[1]

The parasites of human beings include members both of the animal and of the vegetable kingdoms. The animal parasites belong to the divisions protozoa, worms, and arthropods; the vegetable parasites, to the bacteria and fungi. The term pathogenic microorganisms, according to common although somewhat arbitrary usage, includes all of the vegetable parasites and only the protozoa among the animal parasites.

The infectious diseases are caused by pathogenic microorganisms. The special characters of the microorganisms which cause the infectious diseases considered in this work are described in the articles treating of these diseases.

It is thought desirable to bring together in a separate article certain general considerations concerning infection. The most important advances in our knowledge of infectious diseases have come from the study of bacteria. Some knowledge of the general biology of bacteria is essential to an understanding of pathogenic bacterial species and of their relations to disease.

The most important protozoa at present known to be concerned in the causation of human diseases are the malarial plasmodium and the dysenteric amoeba. These are described in connection with their respective diseases. An account of the general biology of protozoa, although of increasing interest and importance to human medicine, would not in the present imperfect state of our knowledge of this subject materially further the aim of this article.

Pathogenic fungi are of comparatively little importance to internal human medicine.

General Biology of Bacteria

MORPHOLOGY AND CLASSIFICATION

The name "bacteria," signifying *staves,* is a convenient but etymologically incorrect one for the group of microorganisms also called or included under schizomycetes or fission fungi, schizophytes, microphytes, protophytes, and, in common parlance, microbes or germs.

The chief reason for regarding bacteria as members of the vegetable kingdom is that they are believed to have more morphological and physiological properties in common with the simplest undoubted plants than with the lowest animals. They are more closely allied to the lower algae called schizosporeae or schizophyceae than to the true fungi.

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[1] In: Theory & Pract. M. (Pepper), Phila., 1894, II, 1-69.

Bacteria are minute, achlorophyllous,[2] unicellular plants which multiply by repeated binary fission, and in many instances also by the formation of spores.

Nuclei have not been satisfactorily demonstrated in bacteria. Inasmuch as the staining reactions of the bacterial cell are more like those of nuclei than of protoplasm, it has been suggested that this cell is composed essentially of nuclear substance or of an intimate mixture of nuclear material and of protoplasm. Bütschli and P. Ernst regard as nuclei certain isolated nuclear staining granules or larger bodies which are demonstrable in some bacteria. It is a growing belief that nuclear material is essential to the existence of a living cell capable of reproduction, so that it is probable that bacteria contain nuclear substance in some form.

The bacterial cell is believed to be always enveloped by a membrane. This membrane is considered by some to be an outer condensed layer of protoplasm, and by others to be the innermost layer of a gelatinous capsule which, at least under certain circumstances, envelopes most if not all bacteria. Bacteria which have a readily demonstrable capsule capable of staining by dyes are described as capsulated.

The substance of the bacterial cells, composed, according to Nencki, essentially of an albuminous material called mycoprotein, appears usually uniform and colorless, but it may present various colors, as yellow, red, green, or violet. In some species the cell may contain starch; in the group of sulphur producing bacteria, granules of sulphur; and in some of the higher forms, the cell membrane may be stained various shades of brown by deposits of iron salts. The cell substance, instead of being uniform, sometimes contains clear vacuoles, sometimes is finely or even coarsely granular, and sometimes presents parts or granules staining more deeply than the rest of the cell or of a different tint, and variously interpreted as condensed or retracted areas of protoplasm, as nuclei, as concerned in the formation of spores, and as degenerations. Probably these isolated, deeply staining particles, which form important morphological criteria in some species of bacteria, are not all of the same character and significance.

The bacteria are divided, according to their forms, usually called growth-forms, into spheres or micrococci or cocci, rods or bacilli, and spirals or spirilla.

The cocci (see Fig. 1, *A*) are farther subdivided, according to their arrangement, into diplococci or paired cocci, streptococci or chain cocci (also torula), merismopedia or merista or tetrads or tetragonous cocci (arranged

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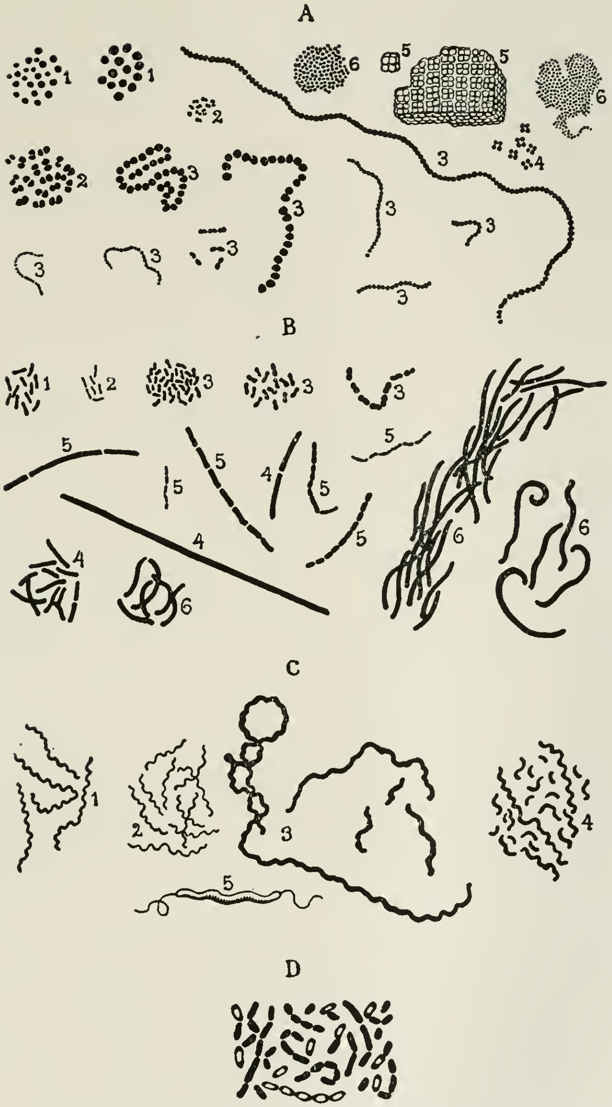
[2] Two or three species of bacteria have been described as containing a green coloring matter believed to be chlorophyl.



Forms of Bacteria: *A*, Micrococci; *B*, Bacilli; *C*, Spirilla; *D*, Bacilli containing spores, (Baumgarten): A^1, single, large and small cocci; A^2, large and small diplococci; A^3, streptococci of different sizes; A^4, *Micrococcus tetragenus;* A^5, *Sarcina ventriculi;* A^6, staphylococci; B^1, rather thick bacilli; B^2, slender bacilli; B^3, dividing bacilli—dumb bell or figure-of-8 shapes; B^4, long bacilli; B^5, bacilli in chains; B^6, curved and wavy bacilli; C^1, *Spirillum obermeieri;* C^2, *Spirochaete dentalis;* C^3, Deneke's spirillum; C^4, *Spirillum cholerae asiaticae*, (comma bacilli and spirilla); C^5, *Spirillum volutans* (vibrio) with flagella; *D*, *Bacillus subtilis*, containing spores, appearing as clear spaces. All (except A^5 and C^5) magnified 950 diameters.

in tablets or in fours), sarcina or packet cocci (of eight or more cells), and as cocci or clumps of cocci enclosed in dense capsules. These differences in arrangement can be explained by the varying direction of the planes of division of the cells and by the separation or the juxtaposition of the cells after division.

All straight bacteria (see Fig. 1, *B*), in which one diameter is greater than the others, are called bacilli, with the exception of those cocci which may have assumed an oval or elliptical shape, usually just before division into spheres. There are forms of bacteria on the border line between rods and spheres, which some prefer to call cocci and others bacilli, but the tendency is to call even the very short oval forms bacilli, which were formerly often designated as cocci.

The old division of rod bacteria into bacteria proper, or short rods without formation of spores, and bacilli, or longer, spore bearing rods, is now generally abandoned, although the name "bacterium" with a distinctive epithet, such as *Bacterium coli commune,* is still often used to designate certain species. Bacteria in the form of long threads are often called leptothrix. Bacilli in chains are sometimes called strepto-bacilli. The name "clostridium" is applied to a spindle-shaped bacillus.

Cohn's division of spiral bacteria (see Fig. 1, *C*) into spirilla, or rigid, spirally-twisted forms; vibrios, with sinuous, low, less pronounced twists; and spirochaete, with long, flexible, corkscrew curves, is now generally abandoned, and the name "spirilla" is used for the whole group, although usage still often retains the terms "vibrio" and "spirochaete" with distinctive epithets, such as *Vibrio metchnikovi* and *Spirochaete dentalis,* for certain species. Singly-curved bacilli are called comma bacilli. Many of these grow into regular spirilla, and they are therefore better designated as spirilla. The spirillum of Asiatic cholera is often called the cholera bacillus or the cholera vibrio.

The botanists usually rank among the bacteria or schizomycetes certain forms which are larger and more highly differentiated than those already described, and which may show an appearance of branching, a distinction between base and ends of the filaments, and considerable variety of shapes even in one and the same species. Most of these, such as cladothrix, crenothrix, and beggiatoa, occur in water. Actinomyces is believed to belong to the group of more highly organized bacteria. These highly differentiated forms are not, however, recognized as bacteria by all authorities, and certainly they should be separated from the other bacteria.

In old cultures and in unfavorable media many bacteria may present various anomalous shapes, such as swellings, varicosities, pear-shapes, twists, bends, and greatly elongated as well as abnormally short or disintegrated forms. These are spoken of as involution forms. Some of these forms may, however, be seen in vigorous fresh cultures of certain species, such as *Bacillus diphtheriae,* and here it does not seem proper to regard them as involution or degeneration forms.

There has been much controversy as to constancy of form among bacteria. As regards this feature, bacteria may be classed as monomorphic, or constant in form; oligomorphic, or variable in form within relatively narrow limits; and pleomorphic, or capable of assuming the various growth-forms of rods, spirals, and spheres. Most of the pathogenic bacteria are monomorphic or oligomorphic; *Bacillus proteus* and many of the highly organized bacteria are pleomorphic. By changes in environment, especially in the composition of culture media, many bacteria present considerable diversity in form, but this diversity relates especially to variations in length and thickness and to the occurrence of involution forms.

Many bacilli and spirilla are motile, whereas only two or three species of cocci are known to be motile. This motility is due to the presence of delicate hair-like flexible processes called flagella, which can be stained by special methods, but otherwise, except with the larger spirilla, are usually invisible. There may be only a single flagellum at one end of the cell, or flagella at both ends, or flagella also along the sides.

Many species of bacteria under certain definite conditions form spores. High temperature, free supply of oxygen, and exhaustion of the supply of nutriment are regarded as favorable to spore formation, but, at least for many species, these conditions are not essential. Endogenous spore formation (Fig. 1, *D*) is that mode which has been longest known and is best understood. It is not known to occur in cocci, is infrequent in spirilla, but is common in bacilli. There is first developed from the protoplasm of the cell a small granule which grows into a sharply circumscribed, highly refractive round or oval body, situated usually either in the middle or in one end of the cell, which it may cause to bulge at the site of formation, and from which it is set free by disintegration of the mother cell. Only one spore is formed, as a rule, in a single bacterial cell. By the ordinary methods of staining bacteria the spore usually appears colorless, and in aqueous media refractive, but it can be stained by special methods. Spores are characterized by greater resistance to heat, desiccation, and other injurious influences than are the vegetative bacterial cells. They consist of an outer dense envelope called the exosporium, and an inner condensed protoplasmic and probably also nuclear material called the endosporium. Placed under suitable conditions, the spore germinates by an outgrowth of the endosporium, in some species through the ruptured ends, in others through the sides of the exosporium.

In distinction to endospores, arthrospores have been described by De Bary and Hueppe. These arthrospores are represented by entire bacterial cells which, it is claimed, have been transformed into bodies with the resisting properties of spores. Cocci, according to this view, may form arthrospores. De Bary and Hueppe have attempted to classify bacteria according to their mode of spore formation, but this attempt has not met with much acceptance. In fact, the whole doctrine of arthrogenic formation of bacterial spores is obscure, and does not, at present, seem to rest upon any certain foundation.

Masses of bacteria held together by sticky intercellular substance are called zoogloea. Masses produced by the growth of bacteria upon solid media are called colonies, the distinctive characters of which form one of the most important means of differentiating bacterial species.

It is now recognized by biologists that a specific systematic classification of plants and animals should be based as little as possible upon physiological differences or differences in function. Hence the higher plants and animals are now classified chiefly according to their structure and development. It is impossible with our present imperfect knowledge to furnish a systematic classification of bacteria based upon these morphological principles, and therefore analogous to the classification of the higher orders of plants and animals. We make as much use as possible, in attempting to classify bacteria, of morphological resemblances and differences, but we are obliged to take into account all of the other properties, including differences of function. The definition of any given bacterial species amounts, therefore, essentially to an enumeration of all of its known properties, such as its form, its origin, its staining reaction, its chemical products, its behavior in a variety of culture media and in the living body. The name given to the species is usually the name of the growth-form, such as micrococcus, bacillus, spirillum, with some distinctive epithet intended to indicate one or more characteristic properties, such as color production, source, arrangement, power of causing fermentation or putrefaction, name of discoverer, cultural peculiarity, or, especially in the case of pathogenic bacteria, the disease caused by the bacterium; for example, *Staphylococcus pyogenes aureus, Bacillus coli communis, Micrococcus tetragenus, Bacillus butyricus, Bacillus saprogenes, Spirillum obermeieri, Bacillus fluorescens liquefaciens, Bacillus anthracis.* Under these circumstances it is evident that what is described and supposed to be a species may subsequently prove to be a race or variety of some species, or, on the other hand, may correspond to a group of species. Notwithstanding these difficulties, and in spite of our inability to define bacterial species in terms analogous to those used for the higher plants and animals, it can scarcely be doubted that bacteria are divisible into distinct species just as much as are the higher organisms.

The present classification of bacteria therefore amounts to little more than a grouping of the many bacterial species under their growth forms, micrococcus, bacillus, etc., with subdivisions relating to biological or chemical properties, such as power of liquefying gelatin, color production, ability to bring about fermentation, pathogenic power, etc. The attempts which have been made to conform to the classification of the higher plants by grouping together in a table species of bacteria into genera and genera into families have not been thus far sufficiently successful or of enough practical utility to make it worth while to consider them here.

FOOD; VITAL MANIFESTATIONS

The capacity of rapid multiplication by division is one of the most striking properties of bacteria. Under favorable circumstances a bacterial cell has been known to divide into two within the space of twenty minutes. If multiplication at this rate proceeded unchecked, the progeny of a single bacterium would be enough in a few days to fill up all the oceans of the world. A check is soon put to this unlimited growth by lack of food, accumulation of bacterial products, and by various other influences, which will be hereafter described, hostile to the multiplication of bacteria.

The food of bacteria, like that of all living things, must contain carbon, nitrogen, oxygen, hydrogen, and certain mineral salts. In general, the most suitable food for bacteria is furnished by the diffusible albuminous substances and the carbohydrates, and these, therefore, constitute the chief ingredients of our artificial culture media. Bacteria, however, differ widely as to their demands for food. Some are capable of obtaining their nitrogen from ammonium salts, nitrites, and nitrates. Being destitute of chlorophyl, they are incapable (with possibly a few exceptions) of assimilating carbon dioxide, but many can obtain their carbon not only from starch and sugars, but also from many organic acids, glycerin, and other alcohols. Hydrogen and oxygen are obtained partly from the same sources as the nitrogen and carbon, and partly from water and free oxygen.

Many pathogenic bacteria require albuminous food, and some require this to be of such special quality that they will not grow in our artificial media, as, for example, *Spirillum obermeieri* and *Bacillus leprae.* At the other extreme are the so-called water bacteria, which Bolton found would multiply with the slight trace of organic material present in ordinary distilled water.

Most bacteria prefer an alkaline reaction of the medium in which they grow, but many will grow in neutral, not a few in acid media, and some even better in acid than in alkaline media.

A certain amount of moisture is essential for bacterial growth. In general, bacteria grow best when their food is not too concentrated.

The temperature limits within which bacteria may develop differ widely, not only for different species, but often also for the same species. The optimum temperature for most pathogenic bacteria is between 30° and 40° C. Usually bacterial growth ceases below 5° to 10° C. (for many pathogenic bacteria at a much higher point) and above 45° to 50° C., but bacteria have been described capable of some multiplication at freezing temperature, and others at temperatures between 60° and 70° C.

Many bacteria require free oxygen for their development, and are therefore called obligate aerobes; others will grow only in an atmosphere free from oxygen, and are called obligate anaerobes; and still others can develop either with or without free oxygen, and are called facultative anaerobes or facultative aerobes. Most pathogenic bacteria are facultative anaerobes; some are obligate anaerobes.

Bacteria by their growth in various media manifest functional activities, some of which are of little significance, but some are of the highest importance in the economy of nature, in the preparation of food, in the industries, in hygiene, and in pathology.

Bacteria are essential for the continuance of life on this globe by their work in decomposing dead vegetable and animal substances into water, carbon dioxide, and the simple nitrogen compounds which form the food of the higher plants and without which they would perish.

The processes of nitrification which take place on a vast scale in the soil are due to the activity of the so-called nitrifying bacteria, of which there are two kinds—one to decompose ammonia into water and nitrous acid, and the other to transform nitrous acid into nitric acid, the resulting nitrates forming the nitrogenous food of chlorophyllous plants. There are denitrifying bacteria which may undo the work of the nitrifying, and reduce nitrates to nitrites and ammonia. The nitrifying bacilli, although devoid of chlorophyl, are capable of forming organic compounds out of purely inorganic matters, and flourish best in media free from organic matter.

The so-called thiogenic bacteria transform sulphuretted hydrogen into sulphur and sulphuric acid.

Among the most important activities of bacteria are those concerned in various fermentations. These fermentative bacteria are called zymogenic bacteria. Those causing the particular kind of fermentation called putrefaction, in which albuminous and other highly organized nitrogenous substances are decomposed with stinking products, are called putrefactive or saprogenic bacteria.

The investigations of Schwann, Schulze, Cagniard-Latour, and, above all, Pasteur, which have led to the establishment of the doctrine that fermentation and putrefaction are due to living organisms, chiefly bacteria and fungi, occupy, together with those inaugurated by Spallanzani concerning spontaneous generation, a very important place in the historical development of the doctrine that the infectious diseases—which, indeed, have been called zymogenic in consequence of their analogies to fermentative processes—are also caused by living organisms.

The study of the various fermentations produced by bacteria and the yeast fungi is of much chemical interest and of great industrial importance, and has become a specialized department of bacteriology which has its own literature.

Here can be mentioned only, as an example of the more important fermentations produced by the yeast fungi, the alcoholic from sugars, and by the bacteria from carbohydrates, the alcoholic, the lactic acid, the butyric acid, the various slimy, viscous, or mucic fermentation, and the fermentation of cellulose with production of marsh gas; the fermentations of the alcohols, of which the most important is the acetic acid fermentation of ethyl alcohol; the fermentations of the fatty acids; the fermentations converting milk into koumyss.

Each species of the zymogenic bacteria is endowed with the power of setting up its own special kind of fermentation, although a single species may be capable of causing different fermentations, or the same variety of fermentation may be produced by a number of species, or in some fermentations two or more species may be concerned simultaneously or consecutively. These fermentations are intimately associated with the vital processes of the organisms producing them, although it is not known in what way, and they cannot be carried to completion by any soluble ferments which have been isolated from the organisms.

Soluble ferments, called enzymes, are, however, produced by bacteria, and doubtless play an important rôle in the metabolism of these lowly organisms as well as in the higher plants and animals. The chemical action of these unorganized ferments is mainly hydrolytic, and does not bring about such complicated changes as do the living bacteria in setting up fermentations. Some of these unorganized ferments have been isolated from cultures of bacteria. Bacteria are capable of producing enzymes with the properties of diastase, of invertin, of pepsin and trypsin, of rennet, of forming ammonium carbonate from urea, and probably also ferments capable of rendering cellulose soluble and of breaking up neutral fats into fatty acids and glycerin. The liquefaction of gelatin, which forms one of the distinguishing cultural characteristics of many bacteria, is a process of peptonization by peptic and tryptic ferments, which, indeed, have been isolated, although not in a pure state, from bacterial cells.

The products of putrefaction are known only in part, but even the known products are very numerous. The simplest end-products of putrefaction are carbon dioxide, water, hydrogen, nitrogen, and ammonia, to which may be added nitrites and nitrates and the gases, sulphuretted hydrogen, phosphoretted hydrogen, and marsh gas. But before the end-products are reached a host of intermediary substances are formed. Among these transitional putrefactive products may be enumerated the following: peptones and other proteid substances; nitrogenous alkaloidal bases called ptomaïns or cadaveric alkaloids, as collidin, neuridin, neurin, cholin, cadaverin, putrescin, spermin, saprin, gadinin, peptotoxin, mydatoxin, mydin, methylguanidin, trimethylamin and other amines; other nitrogenous substances, as leucin, tyrosin, taurin, glycocoll; methylmercaptan; tryptophan; aromatic substances, as phenol, indol, skatol, hydroquinon; and organic acids, as formic, acetic, butyric, lactic, propionic, palmitic, caproic, succinic. Processes of hydration, dehydration, oxidation, and reduction are concerned in the formation of putrefactive products.

Many different kinds of bacteria take part in producing ordinary putrefaction, some aerobic, others anaerobic, one kind preparing the way for the growth of another. The presence or absence of free oxygen has marked influence upon the course and products of putrefaction. It is especially the anaerobic bacilli which produce the most malodorous substances and gases. The course and products of putrefaction are manifold, and vary with the nature of the material, with the temperature, the access of oxygen, and other external conditions, and especially with the character of the microorganisms present.

In considering subsequently food as a source of infection and intoxication attention will be given to the poisonous ptomaïns of putrefaction. The epithet aerogenic is applied to bacteria which produce gas.

Among the vital activities of bacteria may be included the production of pigments. The epithet "chromogenic" is applied to these color producing bacteria. Most of them are harmless. *Staphylococcus pyogenes aureus, Bacillus pyocyaneus,* and a few others are pathogenic. All of the colors of the spectrum, as well as black, are represented in the different tints which may be formed by chromogenic bacteria. Whether the pigment is in the protoplasm or the enveloping membrane of the bacterial cells or in the surrounding medium cannot always be determined, but that it may be in the medium, and even diffused a wide distance from the bacterial growth, is apparent in the cultures of some chromogenic bacteria. As a rule, only the part of the culture exposed to the air presents the color, but with some species it may be in the deeper parts, and even absent from the exposed surface when present in the depth.

Several interesting species of bacteria capable of producing light, and therefore called photogenic or phosphorescent bacteria, have been obtained from decomposing phosphorescent fish and meat and from sea water.

The power of producing disease which characterizes the pathogenic bacteria is a manifestation of vital activity which will be most conveniently considered subsequently in connection with infection.

Here will be explained only the meaning of the terms "saprophyte" and "parasite." A parasite is an organism which lives in or on another living organism, called the host. The name "parasite" is often restricted to such organisms as live at the expense of their host, and the name "commensal" or "messmate" is applied to an organism which lives simply as a tenant or coinhabitant, but not at the expense—and it may be even for the benefit—of the host. The latter mode of life is called symbiosis. The term "saprophyte" was originally applied to plants which grow on decaying vegetable and animal matter, but in bacteriology it designates all bacteria which grow outside of a living host. An obligate parasite is an organism which develops only within a living host. An obligate saprophyte is one which grows only outside of a living body. A facultative parasite is one whose ordinary mode of life is saprophytic, but which is capable of a parasitic existence. A facultative saprophyte is one whose ordinary existence is parasitic, but which can grow in dead organic matter. The bacillus of leprosy, the spirillum of relapsing fever, are, so far as their life history is known, obligate parasites; the bacilli of anthrax, tetanus, and malignant oedema are facultative parasites. The great majority of bacteria are obligate saprophytes. We may by special methods be able to cultivate in our artificial media parasitic bacteria, as, for example, *Bacillus tuberculosis,* which we suppose cannot find, or at least only exceptionally can find, natural conditions suitable for growth outside of the living body; and such bacteria are generally regarded as obligate parasites. No sharp distinction, therefore, can be made between obligate parasites and facultative saprophytes. For some parasitic bacteria we cannot say whether their natural habitat is inside or outside of the body.

DISTRIBUTION

Bacteria are almost universally distributed in the upper layers of the ground, in surface waters, in the air, and on objects exposed to the air. They are especially numerous where there is dead organic material. Only a small fraction of these widely distributed germs are pathogenic. Most of them are harmless and many are useful.

Bacteria cannot ordinarily find suitable conditions, especially the requisite moisture, for multiplication in the air. They are wafted by the air from the ground and objects upon it, but it is a fact of fundamental hygienic importance that the air is incapable of detaching bacteria from moist surfaces except occasionally in spray. Hence only such germs as can withstand complete desiccation are found in a living state floating in the air. Whatever creates dust brings bacteria into the air. The air in the neighborhood of human habitations is particularly rich in bacteria and fungus-spores, whereas sea air at a considerable distance from land is nearly or quite free from microorganisms, this distance naturally varying with the direction and strength of the wind. The air at high altitudes may be nearly or quite free from germs. Bacteria do not seem to occur to any extent in the air as single, detached cells, but rather as clumps or attached to particles of dust, so that in a perfectly quiet atmosphere these particles containing bacteria rapidly settle upon underlying objects, and are easily filtered out by passing the air through porous substances, such as cotton wool, or sand.

The ground, unlike the air, is the resting or the breeding place of a vast number of species of microorganisms. Instead of a few bacteria and fungi in a litre, as ordinarily with the air, most specimens of soil contain thousands, and often hundreds of thousands, of bacteria in a cubic centimetre. Fränkel found the virgin soil almost as rich in bacteria and fungi as that around human habitations. The principal home of the bacteria of the outer world is in the ground. It is here that they live and flourish and carry on those great processes of putrefaction, fermentation, and nitrification so essential to the economy of nature.

This vast richness in microorganisms belongs, however, only to the superficial layers of the earth. Where the ground has not been greatly disturbed by human hands there is, as a rule, at about three to five feet below the surface, an abrupt diminution in the number of living organisms, and at the depth where the subsoil water usually lies bacteria and fungi have nearly or entirely disappeared. Of course the number of bacteria and the depth to which they penetrate vary with the character, especially the porosity, of the soil and the way it has been treated.

Among the pathogenic bacteria which have their natural home in the soil, the most widely distributed are the bacillus of malignant oedema and the bacillus of tetanus. In infected localities *Bacillus anthracis* lives in the soil. Mention has already been made of the presence and function of nitrifying bacilli in the ground.

As regards the presence of bacteria in water, an important distinction is to be made between surface waters and subsoil waters. All surface waters contain bacteria, their number depending upon various circumstances, and especially upon the extent of pollution of the water with animal and vegetable waste. The bacterial flora of water includes a great variety of species. On the other hand, water coming from the deeper layers of the soil is under ordinary circumstances and in most places free from microorganisms, so that such water forms the safest source of supply for drinking water, provided care be taken that it is not exposed to contamination. There are certain bacteria, particularly the so-called water bacteria, which can multiply actively even in the purest water, but this is not true of pathogenic bacteria, which require more concentrated nutriment and are crowded out by the water bacteria. Nevertheless, such pathogenic germs as the bacillus of typhoid fever and the spirillum of Asiatic cholera may survive sufficiently long in water to render this medium an important distributor of these germs to human beings.

The so-called self-purification of water is due to various circumstances, among the more important of which are the action of sunlight and oxygen, the action of bacteria, algae and other organisms, sedimentation and dilution.

As might naturally be expected, microorganisms are present on the skin and exposed mucous surfaces of the animal body, where they are brought by the air and by contact with external substances. Bacteria are carried in large number into the alimentary canal with the food and drink, and are freely inspired with the air. All sorts of bacteria are brought in this way into contact with the skin and exposed mucous surfaces, but most of them are entirely harmless, and are sooner or later removed. There are, however, certain bacteria which are found so constantly or with such frequency in these situations that we can properly speak of a definite bacterial flora of the skin, of the mouth, of the nose, of the intestine, and of the vagina. Thus, in the epidermis and the glandular appendages of the skin we find regularly *Staphylococcus epidermidis albus;* in the mouth, leptothrix and spirillar forms which will not grow on our ordinary culture media; in the intestine, *Bacillus lactis aerogenes* and *Bacillus coli communis;* and in the normal acid secretion of the vagina, *Bacillus vaginalis.* Other species are usually mingled with these regular inhabitants, among the most common and important intruders being the pyogenic cocci, and in the mouth also *Micrococcus lanceolatus.* When the vagina contains pyogenic cocci, its secretion is generally altered in reaction and appearance.

These ordinary surface bacteria cannot in health, with intact skin and mucous membrane, penetrate into the circulation and the internal organs, or if now and then they do happen to make their way into the circulation, they are in health quickly destroyed. The internal organs and fluids are normally entirely free from microorganisms.

AGENCIES INJURIOUS TO BACTERIA

Injurious agencies, according to their nature, duration and intensity of action, may affect bacteria in various ways. Thus bacteria may be killed outright or stopped in development, or manifest only slow, tardy, and scanty growth, or lose or suffer modification of biological properties, such as the capacity of producing fermentation or disease. The modifications of biological properties will be considered separately.

As has already been mentioned, spores are much more resistant to injurious agencies than are the vegetative bacterial cells, although there are decided differences in the resisting power of the spores of different bacterial species, as well as of the different bacteria in the vegetative state.

Bacteria may fail to grow or may develop only feebly or with involution forms, or be otherwise modified in character, when their nutritive pabulum is insufficient or too concentrated or of improper reaction or of unsuitable composition. In our artificial cultivations we endeavor to provide for each bacterial species that nutriment which offers the best conditions for its development. Whereas many bacteria will grow with almost any kind of food we offer them, others, such as the nitrifying bacilli and certain pathogenic bacteria, require nutriment of a very particular composition and of definite reaction. Absence of sufficient water is one of the most common conditions which hold in check bacterial growth, and this property is utilized in preserving foods by addition of sugar and of salt, by drying and by various other processes of concentration.

The cessation of the growth of bacteria in nutritive media may be due to the exhaustion of nutritive supply, but in our culture media it is more frequently the result of alteration in the reaction of the medium and of the accumulation of injurious bacterial products. Bacteria may produce various substances which in sufficient concentration are inimical to their own development or to that of other species; and some of these bacterial products are among the well-known antiseptic substances, such as various acids and phenol. On the other hand, some bacterial products, at least in moderate concentration, are distinctly favorable to the growth and functional manifestations of the bacteria forming them or of other bacteria.

Under natural external conditions bacteria are not usually found in pure culture, but are mixed together, and it is therefore important to know how one species may act upon another. These mutual relations are undoubtedly of a complicated nature, and are only imperfectly understood. The struggle for existence among these lowly organisms must often be keen. We have evidence that one kind of bacterium may by its vigor and rapidity of multiplication simply overgrow and crowd out another; that one kind may produce substances hostile to the development of another; that two or more species may grow just as well or even better side by side; that one species may render the soil more suitable for the simultaneous or subsequent growth of another; that two kinds may grow together, but with modification of the properties of one or both—for example, attenuating or increasing pathogenic power. Such mutual antagonisms and friendships as these must evidently play an important, although at present little understood, rôle in bacterial life.

Bacteria have their natural enemies, not only among their own kind, but also among other low organisms, as well as in the higher plants and animals.

With the exception of the limited, but to us most important, group of parasitic bacteria, these microorganisms cannot live in the living bodies of plants and animals. The power to resist putrefaction has often entered into definitions of life. The inability of bacteria to develop in plants is usually attributed chiefly to the acid reaction of the vegetable juices and to impenetrable vegetable membranes, but it may well be doubted whether the natural immunity of plants to bacterial growth is not due also, in large measure, directly to the vital properties of the vegetable cells. Certain it is that in animals, aside from certain mechanical defences, which will be described hereafter, the living cells of the body furnish the chief weapons to attack invading bacteria; and this is true whether the field of battle be within the bodies of the cells or in the tissues and juices outside of the cells. These antibacterial properties of the cells and fluids of the living animal bear directly upon questions of infection and immunity, and will therefore be more fully considered subsequently.

In this connection the influence of various physical agents upon bacteria may be considered.

Direct sunlight, independently of the heat which it develops, is a powerful destroyer of many bacteria. This action is most intense when the bacteria are in a moist condition, with free access of oxygen and directly exposed to the sun's rays, as, for example, when the bacteria are suspended in water or in a thin layer. Under these circumstances Buchner found water containing 100,000 colon bacteria to be free from living bacteria within an hour. In a few hours, or even less time, direct sunlight may kill anthrax bacilli and spores, tubercle bacilli, typhoid bacilli, cholera spirilla, and many other species. On the other hand, there are bacteria which are not destroyed, or even injuriously affected, by light, and which seek the light. The most decided injurious effect is produced by rays from the violet end of the spectrum. Electric light and diffuse daylight are also injurious to many bacteria, but in far less degree than direct sunlight. Direct sunlight, accompanied often with desiccation and elevation of temperature, is one of nature's most potent agents for destroying bacteria.

Electricity appears to damage bacteria chiefly by its electrolytic action.

Most vegetative bacterial cells are killed by exposure in the moist condition for fifteen to twenty minutes to a temperature of 60° C.; not a few are killed within this period by temperatures varying between 52° and 60° C.; but some require higher temperatures (the tubercle bacillus 65° C. for fifteen minutes), and rare species are known which will even multiply at 60° C. and over. The pathogenic spores, so far as known, with the exception of those of symptomatic anthrax (Rauschbrand), are killed in a few minutes by boiling, but there are very resistant spores which will withstand boiling for five or six hours. All spores are destroyed within twenty minutes by steam under pressure at 120° C.

Nearly all vegetative bacteria are killed in an hour and a half by dry heat at a temperature of 120° C., but the destruction of spores within this period by dry heat may require temperatures of 140° to 160° C.

Some species of bacteria are killed in a few days by freezing, but many survive for a long time. Prudden found alternate freezing and thawing more injurious than continuous freezing.

The cholera spirillum is the most susceptible of all known bacteria to drying, being killed in a few hours by complete desiccation, whereas other vegetative bacteria may survive in the dried state for weeks and months, but are eventually destroyed. Dried spores may retain their vitality for years, and some probably for centuries.

Vibration, such as can be produced by shaking, is favorable to the development of some bacteria, and is injurious to others.

Bacteria may be killed by subjection to high atmospheric pressure—*Bacillus pyocyaneus,* for example, by a pressure of fifty atmospheres of CO_2 in six hours, the anthrax bacilli by twelve atmospheres.

An interesting property of motile organisms was discovered by Pfeffer and called by him chemotaxis. Many motile microorganisms, including bacteria, are attracted or repelled by definite chemical substances dissolved in water. The name "positive chemotaxis" is given to the phenomena of attraction, and negative chemotaxis to those of repulsion. Thus typhoid bacilli and cholera spirilla moving freely in a fluid medium will be attracted into an open capillary tube containing potato juice or solution of potassium chloride placed in the fluid. On the other hand, they will flee from many acids, alkalies, and alcohol, and to other substances they may be indifferent. The phenomena of chemotaxis cannot be explained by simple physical laws, but relate to the life and motility of organisms.

For the antiseptic and disinfectant uses of various chemical substances, as well as for further information concerning heat as a disinfectant, the reader is referred to the section on disinfection in the article on "Hygiene" in "Theory and Pract. M." (Pepper), Vol. I.

MODIFICATIONS OF CHARACTERS; ATTENUATION OF VIRULENCE

In a broad sense bacterial species breed true and transmit the ancestral characters through an indefinite number of generations. But, like other organisms, and apparently in more marked degree than is the case with higher plants and animals, bacteria are susceptible of variation, especially with changed conditions of environment. Also, as with the higher forms of life, some species are tenacious of their special characters, while others are more readily susceptible of variation.

Most of the variations with which we are familiar are temporary, and soon disappear after a return to the normal environment, but some become permanent and heritable even after such return, and thus give origin to new varieties, races, or species.

Permanent variations in function occur more frequently and are more easily produced than permanent variations in form. With our present crude classification of bacteria these functional variations often necessitate the establishment of new varieties which would not be recognized as such if we had a more natural classification.

Most, although not all, of the modifications of character which we can produce are referable to injurious influences, such as prolonged saprophytic cultivation of parasitic bacteria, especially when exposed to light and oxygen, scanty or unfavorable food, high temperatures, prejudicial chemical substances, particularly acids and dilute antiseptics, association with other bacteria or their products, the action of certain animal juices, and passage through the bodies of certain animals.

The most common changes in form under these unfavorable circumstances are those which have already been referred to as involution or degeneration forms, and which are to be regarded as pathological. These disappear under more favorable surroundings.

Some bacteria may present considerable diversity in form, particularly in size, when grown in different media, but there are no well-authenticated instances known in which any material change in form has been permanently established, so as to be long retained with return to the most favorable surroundings. When these most favorable surroundings are to be found in parasitic life, and the bacterium has been deprived of virulence, it may not be possible to restore the usual form; for instance, *Micrococcus lanceolatus,* which grows usually in pairs or short chains, may by prolonged cultivation grow in long chains, and then it has usually lost its virulence.

A functional, rather than a morphological, variation is the loss of the power of developing spores which *Bacillus anthracis* may suffer when cultivated for a long time in repeated generations at 42° C., or when subjected to the action of various injurious chemical substances, as potassium bichromate or carbolic acid. In this way an asporogenic variety of the anthrax bacillus may be produced with permanent loss, even under favorable surroundings, of the capacity to form spores.

The cultural characters of some bacteria have been more or less modified, also the motile capacity of bacteria.

Other interesting functional variations have been produced, such as the loss of the power of forming color, or producing fermentation, or of causing disease. Colorless races of *Bacillus prodigiosus*, of *Bacillus pyocyaneus*, and of other chromogenic bacteria have been obtained and perpetuated. The bacteria of certain fermentations have been deprived of fermentative property by subjection to high temperatures, although in other respects apparently uninjured and capable of vigorous multiplication. The power of peptonizing gelatin has been in the case of liquefying bacteria lessened or abolished.

To us the most important functional variations relate to alterations of virulence of pathogenic bacteria. By subjection to various injurious influences a number of pathogenic bacteria have been made to suffer attenuation or entire loss of their pathogenic power, and in some instances permanent non-virulent varieties have in this way been established.

Bacteria have been deprived partly or wholly of their virulence by prolonged cultivation in artificial media; by injurious physical agents, as heat, sunlight, desiccation, high atmospheric pressure; by antiseptic chemical substances, as carbolic acid, potassium bichromate, trichloride of iodine; by the blood serum of certain animals; by products of certain animal cells, especially those in extracts of the thymus gland; by certain bacterial products, for example, cadaverin and undetermined ones in sterilized cultures; by mixture with other bacteria, as, for instance, the anthrax bacillus with *Bacillus pyocaneus* where also the controlling factor is doubtless the action of chemical products; and by inoculation of certain animals.

The more gradual and the less violent is the operation of these attenuating influences, the more permanent is the resulting modification.

Most bacteria become more or less weakened in pathogenic power by prolonged cultivation in artificial media, especially when exposed to light and oxygen. With some this diminution of virulence may be scarcely perceptible after many months, and even years, but with *Micrococcus lanceolatus* the virulence may have entirely disappeared within a few days.

By cultivating the anthrax bacillus at 42° C. any degree of attenuation of virulence, down to complete loss, may be produced, according to the duration of exposure to this temperature. In this way permanent attenuated and non-virulent varieties of this bacillus may be developed.

In many cases, if not in all, loss of virulence is to be interpreted as loss of power of producing toxic substances. The loss of this function is often, although not necessarily, associated with enfeeblement of other functions, such as the capacity of vigorous and rapid multiplication and of resistance to various harmful influences. Loss of virulence resulting from prolonged cultivation outside the body is generally associated with more vigorous growth, the saprophytic character becoming more pronounced at the expense of the parasitic.

In contradistinction to attenuation, exaltation of virulence may sometimes be secured. This also has been accomplished in various ways, as by the addition of certain chemical substances, by mixture with other bacteria or their products, and especially by repeated passage through the bodies of relatively insusceptible or of susceptible animals.

We have not much information as to the occurrence of natural or spontaneous variations of bacteria independently of such accidental or designed changes in environment as have been mentioned. Nevertheless, there is no doubt that such variations occur. They can be observed especially well in the case of the apparently spontaneous appearance of colorless varieties of chromogenic bacteria. It has been shown that in one and the same culture of the anthrax bacillus and in that of *Micrococcus lanceolatus* the bacteria may be possessed of different degrees of virulence. Under certain conditions, by a process of natural selection, one or the other of these natural varieties may gain the upper hand and displace the rest, but this explanation, although it has been advanced, is not generally applicable to the various artificial methods of attenuating pathogenic bacteria. We meet under natural conditions both nonvirulent and virulent *Bacilli diphtheriae* and *Micrococci lanceolati,* and we find the pyogenic cocci notably variable in virulence. Natural variations have been observed also with the cholera spirillum, the typhoid bacillus, and the colon bacillus.

The question of the mutability or the fixity of the varieties and species of bacteria has been much discussed. The facts described in this section evidently bear upon this question, but, with our present knowledge, it is not clear just how much weight should be attached to them in answering the question. Doubtless some writers have exaggerated, and others have underestimated, their significance. The fact that most of the modifications, at least those of a permanent character, relate to an increase or diminution of certain functions, and that many are clearly degenerative, would lead us to attach relatively small importance to them in the differentiation of species if we were to be guided, as, unfortunately, we cannot be at present, by the principles employed in determining species in the higher plants and animals. Even if we assign to these modifications the fullest admissible weight, and if we admit that bacterial species and varieties are more liable than those of higher organisms, there is nothing which would justify us in relinquishing the opinion that bacteria are divisible into definite species and varieties, or in supposing that one well-established species can be transformed into a different, already existing, well-established species, as, for instance, *Bacillus subtilis* into *Bacillus anthracis,* or *Bacillus tuberculosis* into the bacillus of glanders. New species of bacteria have been evolved, doubtless, by the same laws of variation and of natural selection which prevail throughout the animal and vegetable kingdoms. From a medical point of view the occurrence of natural variations in the pathogenic powers of bacteria is clearly of great importance in interpreting the varying characters of epidemic diseases.

MARKS OF DIFFERENTIATION

For the differentiation of bacteria it is necessary to make as complete a study as possible of all their characters, morphological, biological, and chemical. These characters are of varying degrees of diagnostic importance, but even slight and apparently trivial differences may prove to be of significance. Very few bacteria can be positively identified by their morphological characters alone. To these characters must be added, for purposes of diagnosis, a larger or smaller number of other characters, such as staining reactions, cultural properties, chemical products, and pathogenic power. With increasing knowledge of new bacterial species it often happens that marks which were at first thought to be sufficient for the identification of a species are found not to suffice for its separation from other species. Bacteriological literature contains a large number of named bacteria which have been so imperfectly described that nobody could identify them from the description, and there is no doubt that the same species often figures under different names or that the same name has been assigned to different species. This leads to no little confusion, and shows that it is the duty of every one who brings before the world a new species and names it to describe it in all its characters as fully as possible.

The description of the methods employed in studying bacteria belongs to bacterial technology, which does not come within the scope of the present article. It is proper, however, to enumerate here the differential characters which, so far as possible, should be determined for each species or variety of bacteria.

1. Source. Natural habitat, as ground, water, animal body.
2. Morphology. Everything pertaining to size, form, and development, including involution-forms and other variations of form, grouping, as in pairs, chains, clumps, appearance of zoogloea, presence of capsules, development and germination of spores, arrangement of flagella.
3. Staining properties, especially reaction with Gram's (or Weigert's fibrin) stain, and peculiar or irregular staining.

4. Motility, especial care being taken to examine very fresh cultures and cultures in different media.

5. Temperature at which multiplication occurs, including optimum, maximum, and minimum temperatures.

6. Relations to oxygen, whether aërobic, anaërobic, or faculative anaërobic. Relations to other gases, as hydrogen, carbon dioxide, may also be determined.

7. Reaction of media in which growth occurs, and changes in reaction produced by the growth.

8. Cultural characters, in bouillon, nutrient gelatin, nutrient agar, potato, milk, blood serum. The composition of the culture medium may be modified in various ways for special purposes, as by the addition of litmus, sugar, dyes, and other reagents, and additional media may be employed. The points to be noted are too numerous to specify, but among those of fundamental importance are whether or not gelatin is liquefied, milk is coagulated, color is produced, a surface membrane is formed on bouillon, and the appearance of the growth upon potato. A precise description of the colonies in nutrient gelatin is of especial importance.

9. Resistance, both in the vegetative and spore state, to heat, desiccation, prolonged cultivation, antiseptic substances, and other injurious influences. It is desirable to fix precisely the thermal death point. The germicidal action of the blood serum of different animals may also be tested on the organism.

10. Powers of fermentation, tested with various fermentable substances, particularly with glucose and lactose.

11. Pathogenic powers. There should be stated the modes of inoculation, quantity inoculated, duration of disease and symptoms, distribution of the bacteria, and the lesions produced; also the animals which are susceptible or immune, and variations in virulence.

12. Chemical products, both those produced in cultures and those formed in the bodies of infected animals. Of the metabolic, fermentative or putrefactive products formed in cultures it is often desirable to determine not only the kinds, but also the amounts, of gases and acids produced, and especially the production of nitrites and of indol as tested by the nitroso-indol (cholera-red) reaction. The detection of specific toxic and immunizing products is of valuable assistance in studying pathogenic bacteria.

General Considerations Concerning Infection

DEFINITIONS

The word "infection" has been and is still used in various senses.

1. By some writers it is used, in distinction from contagion, to indicate the transmission of disease without personal contact, as through the air and

infected things, not persons. This usage, once common in medical writings and still popular, is dying out among physicians, and is no longer supported by the best medical authorities.

2. According to another usage, infection refers primarily to a diseased condition characterized by certain symptoms and lesions, and only secondarily or not at all to the nature and mode of transmission of the morbific agents causing the disease. The etymology of the word—from *inficere,* to taint, to spoil—seems to justify this meaning, but there enters also into the etymological sense the conception of something introduced from without. The morbific agents capable of producing infection in this sense are heterogeneous and not easily classified, including many, but not all, pathogenic bacteria; some, but not all, pathogenic fungi; some, but not all, pathogenic protozoa; a few parasitic worms, notably trichinae; some, but not all, toxic bacterial products; some poisons produced by other vegetable cells, as abrin and ricin; and some poisons produced by animal cells, as snake venom. These poisons, produced by vegetable and animal cells, belong mainly to those called ptomaïns, leucomaïns, and toxic proteids. It will be observed that this second definition of infection is essentially clinical. This use is not common, and is not to be recommended.

3. Modern investigations have shown that the most common and typical infectious diseases, according to any signification of the term infectious, are caused by the invasion and multiplication of pathogenic microorganisms; and it is now customary, at least among bacteriologists, hygienists, and pathologists, to limit the term infection to the morbid condition caused by the entrance and growth within the body of pathogenic microorganisms, and to the act or process by which disease is thus caused. Infection in this sense refers to the nature of the morbific agents causing disease, and implies nothing as to the mode of transmission of these agents. The pathogenic microorganisms which we now know to be concerned in causing infections belong to the classes bacteria, fungi, and protozoa, but we know nothing about the specific agents of infection in many, and these among the most typical, infectious diseases, such as the exanthematous fevers. Infectious diseases, as thus defined, form a tolerably definite and well-recognized group of affections.[3] An infectious disease may be localized or general.

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[3] The not very happy term, at least in its English dress, "invasive diseases" (Invasionskrankheiten) has been employed to designate other parasitic diseases, particularly those caused by entozoa. One is certainly reluctant to exclude trichinosis from the list of infectious diseases, as well as to admit to the list most of the diseases produced in human beings by fungi, such as thrush and the dermatomycoses. This reluctance is due to the fact that we cannot well abandon the conception that infection implies a permeation of the body with microorganisms or their products, such as we generally observe in bacterial diseases. If, however,

According to this third definition, which is the one followed in the present article, infections are distinguished from intoxications. Poisoning with bacterial products plays the chief rôle in producing the phenomena of an infectious disease, and it is impossible to draw any sharp dividing line between intoxication and infection; but it is believed to conduce to precision and clearness to regard as agents of infection only such as are capable of reproduction—that is, such as are living organisms—and not to include among these agents chemical poisons, whether produced by bacteria or other vegetable cells or by animal cells.[4]

The old terms contagion, miasm, and miasmatic contagion no longer answer the purpose of modern medicine, but it is necessary to give the student some idea of the meaning attached to them.

A contagious disease is one capable of communication from one individual to another by means of direct or mediate contact. The introduction of the expression mediate contact in this definition is necessary, but it greatly lessens its precision. In ordinary use the epithet contagious is applied only to those diseases which are readily communicable from individual to individual, so that a person affected with the disease is a source of danger to those in proximity to him. When the infectious agent is eliminated from the

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we take a broad view of the relation of fungi to diseases of some of the lower animals, and especially to plant diseases, we can hardly fail to include pathogenic fungi among infectious organisms.

[4] The difficulties which arise if we include, as is sometimes done, among infectious diseases simple intoxication with bacterial products without the entrance and multiplication of the causative bacteria within the body, are numerous. The bacteria concerned in the not unimportant group of affections caused by the reception of ptomaïns and some other bacterial products formed outside of the body, as in poisoning from decomposing meat, fish, milk, etc., are mostly strict saprophytes, incapable of parasitic growth in the body of the affected animal. It is only certain poisonous products of bacteria which any one would think of considering as infectious agents, and it would not be easy to define which toxic products should be admitted to the group and which excluded. If we admit as infectious agents toxic products of the bacterial cell, there is no reason why we should exclude similar chemical substances, particularly the toxic albumoses, produced by other vegetable cells and by certain animal cells. Poisoning by snake-venom is clinically a typical infection, and is so regarded by Virchow and others, who adopt a clinical rather than an etiological meaning of the word "infection."

If there were any chance, as there is not, of the word "infection" disappearing from medical nomenclature or from popular use, or if there were any good substitute for this word, it would probably not be worth while to try to adapt this old term to our modern conceptions based upon a more exact knowledge of the causation of disease, and it might be left to the vague and loose meaning which has always attached to it in the minds of most people.

diseased body only in such a way, as by the faeces, that it is not likely to be directly received by a second individual, but takes a roundabout way, as though the ground and water, to reach another individual, then the disease is not usually called contagious, although under certain circumstances it may be so. Typhoid fever and Asiatic cholera are examples of such diseases.

It is evident that the term infectious diseases is a more comprehensive one than contagious diseases, and is not strictly comparable with the latter, as the former refers to the nature of the agents causing the disease, and the latter to the manner of transmission of these agents from individual to individual. It is also clear that the limits of what shall be called contagion are indefinite and arbitrary. It is not uncommon to speak of any infectious agent as a contagium. Whether or not an infectious disease is contagious in the ordinary sense depends upon the nature of the infectious agent, and especially upon the manner of its elimination from and reception by the body.

Most, but not all, contagious diseases are infectious. Scabies is a contagious disease, but it is not infectious.

A miasmatic disease is one caused by an ectogenous infectious agent neither directly nor indirectly derived from another case of the same disease. The type of a miasmatic disease in the sense defined is malaria or the malarial diseases. The cause of malaria is a protozöon, which multiplies within the body but, so far as we know, is never eliminated from the body in a living state capable of infecting another individual or the locality. The term miasm is sometimes used in a loose sense to designate infectious agents coming from the water or the ground, especially when believed to be conveyed by the air, or even to designate noxious gaseous emanations and undefined effluvia from the ground.

The expression miasmatic-contagious diseases was introduced in order to explain the often complicated, and even yet not thoroughly understood, modes of spread of Asiatic cholera and typhoid fever, and especially to this group was attached a variety of hypotheses, now for the most part overthrown, and which it is not necessary here to mention. In this group of so-called miasmatic-contagious diseases the infectious agent is eliminated from the diseased body in the living state, but in such a way that it is not likely to be directly received by a second individual, and to produce a second infection it must take ordinarily a path, often circuitous and tortuous and not always easily traced, through various external media. This transit may be influenced, in ways which we now only imperfectly understand, by different meteorological and telluric agencies.

EVIDENCE OF THE CAUSATION OF INFECTIOUS DISEASES BY MICROORGANISMS

The doctrine that infectious diseases are caused by parasitic organisms is centuries old, and, before the discovery of the specific organisms, the evidence in its favor, based mainly upon *a priori* reasoning and analogy, was presented during the first half of this century with great clearness by J. K. Mitchell in this country and in Europe by Holland, Eisenmann, and, above all, Henle. The property of indefinite multiplication which belongs to infectious agents was the strongest point in this logical argument, as we cannot attribute this property to anything except a living organism. The evidence now, however, for many infectious diseases is no longer aprioristic, but is based upon incontrovertible observations and experiments relating to the causative microorganisms. We owe to Pasteur as the pioneer and then to Koch and to their followers, the great mass of this positive evidence, which has introduced a new era in the history of medicine.

The complete chain of evidence, essentially as formulated by Koch, to demonstrate that a disease is caused by a specific microorganism, is as follows:

a. The presence of the organism in all cases of the disease and in such distribution as will explain the lesions.

b. The isolation of the organism in pure culture.

c. The reproduction of the disease by inoculation with the isolated organisms.

When all of these conditions have been fulfilled there can be no doubt that the disease is caused by the organism, and, as one of the conditions is the unfailing presence of the same organism in every case of the disease, is caused solely by this organism.

In addition to the points mentioned, important evidence of the causal relation of a microorganism to a disease may be afforded by the study of certain chemical substances produced by the organism both in cultures and in the animal body. Many bacteria exert their pathogenic powers not so much by their mere presence as by toxic substances produced by them or contained within them, and many of these toxic substances have specific properties by which they can be identified as the products of definite species of bacteria. Certain amorphous toxic products of bacteria and the immunizing and curative proteids especially render valuable assistance in demonstrating the etiological significance of bacteria. Most of these substances, unfortunately, cannot at present be defined chemically, but they can be recognized by their biological attributes, as will be explained subsequently.

1. For the diseases affecting human beings the three conditions above laid down have been fulfilled in tuberculosis, tetanus, anthrax, glanders, and probably gonorrhoea. Probably acute croupous pneumonia should also be here included, but it is still claimed by some competent authorities that genuine acute croupous or lobar pneumonia may be caused by other organisms besides *Micrococcus lanceolatus.*

Under the name diphtheria have been hitherto included etiologically distinct affections. Most cases of genuine primary diphtheria are caused by the Klebs-Löffler bacillus of diphtheria. It is probable that the cases caused by this bacillus possess clinical and pathological characters distinguishing them from forms of disease hitherto called diphtheria, but produced by other microorganisms. If we can thus separate as a distinct disease diphtheria caused by the Klebs-Löffler bacillus from other affections which have been called diphtheria, then the former disease is to be included in the group of those in which the three conditions of Koch have been fulfilled. If, however, the name diphtheria continue to be used to designate etiologically distinct affections, then diphtheria will rank with those diseases which can be produced by more than one kind of organism.

2. There is a large class of infections in human beings in which the three conditions of Koch have been fulfilled with the exception of constancy in the presence of the same organism in all cases of the disease. Here also the evidence is conclusive that a specific microorganism, found properly distributed in the disease, isolated in pure culture, and when thus isolated capable by inoculation of reproducing the disease, is a cause of the disease, but it is not the sole specific cause. For the most part, the diseases which can thus be referred to more than one kind of microorganism are the various septic and inflammatory affections, which do not present such sharp and definite differential characters as do those more specific infectious diseases which are caused by only a single species of microorganism. Some of these diseases present clinical and pathological differences in accordance with their etiological differences, whereas others do not. The class of microorganisms chiefly concerned in the etiology of these diseases embraces the somewhat heterogeneous group called pyogenic bacteria, of which the pyogenic staphylococci and streptococci are the leading, but not the sole, representatives. To this class of affections belong acute ulcerative endocarditis, meningitis, bronchopneumonia, erysipelas, pyaemia, septicaemia, osteomyelitis, puerperal fever, other septic and localized inflammatory affections, and, in general, the infections of wounds. Most of these names are simply collective names for different diseases which have already received, and are likely still further to receive, more exact definition as the result of bacteriological studies.

3. The next group of infectious diseases embraces those in which a specific microorganism is found constantly and exclusively associated with the disease, and has been isolated in pure culture, but the disease has not been reproduced experimentally by inoculation of the isolated organism; in other words, diseases in which the first two, but not the last, of the three conditions of proof above mentioned have been met. To this group of diseases belong

typhoid fever, Asiatic cholera, perhaps influenza, rhinoscleroma, actinomycosis,[5] and some of the fungous diseases, which will not here be considered.

Typhoid fever and Asiatic cholera are the diseases which chiefly interest us in this connection. So far as we know, lower animals are not susceptible to either of these diseases. The bacillus of typhoid fever and the spirillum of Asiatic cholera manifest in lower animals interesting pathogenic properties which elucidate many points in the pathogenesis of these diseases, but their inoculation in animals has failed to reproduce satisfactorily a disease corresponding in essential features to that observed in human beings; and, in view of the known insusceptibility of lower animals to these as well as to many other diseases of human beings, this result is not surprising. It is true that we do not usually produce in lower animals by experimental inoculation the exact counterpart of the corresponding disease in human beings, nor is it necessary or to be expected that we should do so in order to rest satisfied with our experimental evidence; but the so-called experimental typhoid fever and cholera of guinea pigs and rabbits are too divergent from the natural diseases in methods of production, in course, symptoms, lesions, and distribution of the bacteria, to be accepted as satisfactory reproductions of these diseases as they occur in man.

Pure cultures of the cholera bacillus have been intentionally swallowed by persons who have previously alkalinized the contents of the stomach with sodium bicarbonate. This experiment has been made in several instances by different individuals. In the majority of cases there resulted diarrhoea of moderate severity, unattended by those marked constitutional symptoms which are attributed to the absorption of the specific poison produced by the cholera spirillum. The cholera spirilla multiplied in the intestinal canal and were found in the faeces. In one case reported by Metchnikoff the ingestion by a healthy young man in Paris of one-third of an agar culture of the cholera spirillum, apparently attenuated in virulence by prolonged cultivation, was followed by a typical attack of Asiatic cholera with all of the classical symptoms—frequent, painless, profuse diarrhoea lasting two days, rice-water stools, repeated vomiting, subnormal temperature, muscular cramps, and anuria on the second day. Reaction began on the third day, and recovery was complete. The diarrhoea set in twelve hours after drinking the culture. During the first two days the intestinal discharges contained an almost pure culture of the cholera spirillum. After the fifth day the cholera spirillum rapidly diminished, but delicate methods revealed a few as late as the seventeenth day. No cholera had existed in Paris for months preceding this experiment. This is the only instance, out of more than a dozen similar experiments, in which a severe attack has followed the intentional ingestion of cholera cultures.

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[5] Wolff and Israel have apparently succeeded in producing actinomycosis by inoculation of animals with pure cultures of actinomyces, so that this disease may perhaps properly be classified with the first group. Further experimental work, however, is needed to thoroughly elucidate this subject.

The results of these experiments on human beings are not necessarily out of accord with observations as to the occurrence of cholera under natural conditions of infection, and do not compel us to suppose that the cholera spirilla are incapable of producing the gravest forms of the disease. Out of a large number of persons exposed to infection with cholera only a portion, usually a minority, acquire the disease. Of the latter only a portion, usually a minority, develop the more severe grades of cholera; others manifest simple choleraic diarrhoea without marked constitutional disturbance, and some may receive the spirilla without experiencing even diarrhoea or other noteworthy symptoms, although the bacteriological examination of their stools reveals the presence of cholera spirilla. In other words, the same infectious material may cause in different individuals all grades of cholera from simple diarrhoea to the gravest algid cholera, or it may even leave the individual unaffected.

We are not informed at present at to the special conditions of individual predisposition or other conditions which enable us to explain this remarkable diversity in the effects produced by the entrance of cholera spirilla into the intestinal canal of different persons.

Both the typhoid bacillus and the cholera spirillum produce specific poisons and immunizing substances, which, as will be explained subsequently, afford significant corroborative evidence that these organisms are the specific cause of their respective diseases.

The following group of infectious diseases affords an opportunity to consider the weight of evidence to be attached to the constant and exclusive association of a specific microorganism with a disease.

4. In this fourth group of infectious diseases only the first of the three conditions of complete and stringent proof has been fulfilled. Here belong leprosy,[6] relapsing fever, and the diseases caused by protozoa, the most important of the protozoan diseases now known to occur in man being malaria and amoebic dysentery. No medium has thus far been found on which the bacillus of leprosy or *Spirillum obermeieri* can be artificially cultivated, nor have we at present any means of cultivating the malarial protozoa and *Amoeba dysenteriae*. Certain affections of lower animals, and possibly some in human beings, caused by other sporozoa may, however, be included in this group, but we shall not consider them here.

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[6] Several investigators claim to have cultivated leprosy bacilli, but in no instance, thus far, has this claim been substantiated to the satisfaction of most bacteriologists.

The constant and exclusive association of a specific microorganism with the lesions of a disease admits of no other interpretation in harmony with our existing knowledge than that the organism is the cause of the disease. The only other supposition which deserves a moment's consideration is that the disease is the cause of the presence of the organism. There are instances in which we know that a disease is the cause of the presence of bacteria, but in these cases the same bacteria are found independently of the disease in which they happen to be present; in fact, these secondary invaders are for the most part widely distributed and common species of bacteria. Moreover, we do not find with these secondary bacterial infections one and the same species constantly present in a given disease. We cannot, therefore, here, as in the class of infectious diseases now under consideration, speak of the constant and exclusive association of one and the same specific microorganism with a given disease. The possibility that a diseased condition may change some entirely different, perhaps saprophytic, bacterium into the species which is found constantly and exclusively associated with this diseased condition is not supported by any established facts of observation or experiment, and is wholly improbable. Again, it is to be urged that whenever we have found a definite bacterial species constantly and exclusively present in the lesions of a disease, and it has been in our power to isolate in pure culture this bacterium, and to inoculate the isolated organism into an animal susceptible to the disease, it has been demonstrated that such bacterium is the cause, never an effect, of the disease.

5. Finally, we have a large number of infectious diseases which have thus far resisted all efforts to discover their specific infectious agents. Here belong yellow fever, typhus fever, dengue, mumps, rabies, Oriental pest, whooping cough, smallpox and other exanthematous fevers, syphilis, and some other infectious diseases in human beings. It will be noted that many of these are the most typically contagious diseases, which it might have been supposed would be the first to unlock their secrets, whereas some of the known bacterial diseases were not previously generally regarded as infectious.

It is, upon the whole, improbable that most of the infectious diseases whose specific microorganisms have been carefully sought for in vain will be found to be caused by bacteria. They are more probably caused by some other class of microorganisms, for the demonstration and study of which we have not at present the same satisfactory methods as for bacteria.

We have had in view in this section chiefly the infectious diseases of human beings. There are many diseases confined to lower animals which have been proven to depend upon bacteria, such as chicken cholera, hog cholera, swine erysipelas (Rothlauf), symptomatic anthrax or black leg (Rauschbrand). Here may also be mentioned malignant oedema, which may occur in human beings as well as in lower animals. Texas fever of cattle has been shown by Theobald Smith to be caused by an organism belonging probably to the protozoa.

GENERAL ETIOLOGY OF INFECTIONS

The mere demonstration of a specific microorganism as the cause of an infectious disease is not a solution of all of the etiological problems belonging to the disease. There are many other etiological factors of importance, some relating to the infectious microorganism and others to the individual exposed to infection. Many of these factors are often called secondary or accessory causes.

Number and Virulence of Infecting Bacteria.—The number and degree of virulence of bacteria received into the body may be determining causal factors. In the case of some pathogenic bacteria a single bacterial cell may be capable of infecting a highly susceptible animal, whereas with a less susceptible animal a large number of the same kind of bacteria may be required. With some, and probably most, kinds of pathogenic bacteria the dosage is an important matter, infection occurring sometimes only when many thousand bacteria are inoculated. This dosage, however, is largely a question of individual or racial susceptibility on the one hand and of virulence on the other. Moreover, the kind of infection produced by some bacteria varies with the dose. For instance, *Micrococcus lanceolatus* may possess such a degree of virulence that a small number of the bacteria inoculated subcutaneously into rabbits will produce no apparent effect, a larger number will cause a local abscess only, and a still larger number will set up fatal general septicaemia.

In our laboratory experiments we are familiar with varying degrees of virulence in the case of a large number of pathogenic bacteria, and, although we have less positive information as to similar variations under natural conditions, we know of many instances of this occurrence—for example, with pyogenic cocci, *Bacillus diphtheriae, Bacillus typhi-abdominalis, Micrococcus lanceolatus, Spirillum cholerae asiaticae, Bacillus coli communis*—and we know that influences capable of affecting virulence, such as sunlight, desiccation, saprophytic growth, association with other bacteria and their products, are operative in nature.

Capacity for Saprophytic Growth.—Microorganisms which are enabled by external conditions to multiply outside of the body evidently increase thereby the chances of infection by a wider distribution and by their greater number. The diseases usually called endemic, which are spread through infection of a locality, such as malaria, anthrax in animals, amoebic dysentery, typhoid fever, and Asiatic cholera, are caused, at least for the most part, by microorganisms capable of saprophytic development.

The more important diseases of human beings caused by microorganisms believed to be obligate parasites are tuberculosis, leprosy, relapsing fever, gonorrhoea, syphilis, rabies, and probably smallpox and other exanthematous fevers. Those caused by organisms whose natural habitat is outside of the animal body (facultative parasites) are malaria, amoebic dysentery, anthrax, tetanus, malignant oedema, actinomycosis, perhaps yellow fever. Those caused by parasites which may multiply outside of the body (facultative saprophytes) are typhoid fever, Asiatic cholera, various affections caused by pyogenic bacteria, and, probably to a less degree, croupous pneumonia and diphtheria. This classification is based partly upon the behavior of the different pathogenic bacteria in our artificial cultures, and partly upon observations concerning the modes of spread of the diseases enumerated. Our knowledge concerning the natural conditions of growth of pathogenic organisms and the sources of infection is not for many infectious diseases sufficiently accurate and extensive to enable us to classify them, except provisionally, under one or another of these heads, and for some we cannot even venture a provisional classification.

Vital Resistance of Pathogenic Bacteria.—The power of causing ectogenous infection is by no means confined to organisms capable of saprophytic growth, upon which, therefore, undue stress should not be laid. Obligate parasites possessing sufficient resistance may live a long time outside of the body and become widely distributed upon external objects. They may even be better adapted for prolonged external existence than some organisms capable of multiplication outside of the body. The tubercle bacillus is an example of such a widely distributed microorganism finding only exceptionally conditions suitable for growth outside of a living host.

Of course the bacteria which form spores are the most resistant. The only bacteria infectious for human beings which are positively known to develop spores are the bacilli of tetanus, anthrax, and malignant oedema, all killed by exposure in the moist condition for a few minutes to boiling temperature. It is generally stated that tubercle bacilli form spores, which are, however, less resistant to heat than the preceding. The claim that the bacilli of glanders, typhoid fever and leprosy also form spores has not been established to the satisfaction of all bacteriologists, and the spores of glanders and typhoid bacilli, if they exist, must be much less resistant to heat than the spores of anthrax, tetanus and malignant oedema. The tubercle bacillus, the pyogenic cocci, and the typhoid bacillus are among the more resistant bacteria which are not proven to form spores.

The vital resistance of microorganisms to desiccation is, as has already been explained, an important factor in determining the mode of transmission of an infectious agent, particularly the possibilities of infection through the air. The speedy death of cholera spirilla by drying virtually excludes the conveyance of these germs by the air.

Portals of Entry.—These are the skin, the exposed mucous membranes, as the air passages and lungs, the alimentary tract, the genital and urinary tracts, and wounds of the skin and mucous surfaces. Intrauterine infection of the foetus will be considered later. In our laboratory experiments we make frequent use of methods of inoculation which occur only exceptionally or not at all under natural conditions, such as injection of virus directly into the vessels, into the serous cavities, beneath the skin, and forced inhalation of large numbers of bacteria. In fact, we can rarely imitate experimentally the precise conditions of natural infection.

Some pathogenic organisms are capable of causing infection only when introduced in a definite way—as, for instance, tetanus and malignant oedema bacilli into wounds, cholera spirilla, so far as human beings are concerned, into the alimentary canal—and most have a distinct predilection for particular portals of entrance, as is evident in syphilis, gonorrhoea, diphtheria, pneumonia, tuberculosis, etc.; but some of these, such as the tubercle bacilli, can invade any channel. For a large number of infectious agents, such as those of the exanthematous fevers and relapsing fever, we have no positive information as to how they get into the body, and are left to more or less probable conjecture.

The inference that advanced lesions produced by a specific microorganism at one or more of the situations above enumerated as portals of entry indicate that the organisms must have entered there from without the body, is a common one, but not always warranted.

It is interesting to consider the defences which have been set up at the different exposed parts of the body.

The skin, which is the part most exposed, is protected by a dense epidermis, impenetrable when intact to ordinary bacteria, including most pathogenic forms. Hence we recognize wounds of the integument, which may be so slight as to be difficult or impossible of detection, as a most important predisposing cause of cutaneous infection. There are, however, pathogenic organisms which may settle in the healthy skin and produce disease, but it is improbable that there are any microorganisms which can pass through the healthy skin and thence invade the body without producing any damage at the point of entrance.

The mucous membranes at the orifices of the body, and some elsewhere, as the buccal, oesophageal, vaginal, which are covered with thick laminated flat epithelium, are hardly less efficiently protected by the nature of their covering than is the skin.

The more delicate mucous membranes covered by cylindrical epithelium are so situated as to be less exposed to insults, but even these apparently more penetrable mucous membranes probably do not permit the passage of many pathogenic bacteria without the occurrence of some damage to their integrity. So far as we can determine, however, some pathogenic organisms—for example, the malarial protozöon—can pass through intact surfaces into the interior of the body. We must admit this for tubercle bacillus, unless we accept Baumgarten's doctrine of inheritance of the bacillus with an indefinite latent period.

There are certain situations, particularly the tonsils and the lymphatic follicles of the intestine, which seem, by the nature of their covering, especially exposed to the invasion of bacteria; but there is reason to believe that this lymphatic tissue is richly endowed with vital properties hostile to the development of most bacteria.

There are in some situations other mechanical protective arrangements besides the thickness of the covering. Ciliated epithelium drives foreign particles toward the natural outlets of the body. Certain canals communicating with the exterior are only occasionally opened, as the urethra and uterus, and the urethra normally under conditions not permitting the entrance of organisms. The bladder and uterus do not ordinarily contain any bacteria. The tortuous arrangement of the air passages, especially in the nose, the fact that air is directly inspired only for a relatively short distance into the air tubes, and the presence of ciliated epithelium are serious obstacles to the penetration of foreign particles into the smaller bronchi and the lungs, so that in these in health bacteria are generally either absent or present only in small numbers, whereas the passages above the larynx contain many organisms.

If we were to rely exclusively upon the results of experiments in the test-tube on the germicidal action of the acid gastric juice, particularly the very acid juice of the dog, we should consider this action a formidable obstacle to the passage of many living bacteria into the intestine. Doubtless this acid fluid is a protective factor not to be overlooked, especially as regards an organism so susceptible to acids as the cholera spirillum; but when we consider the insusceptibility of many bacteria to weak acids; the relatively slight acidity of the human gastric juice, the absence often of any acid in the stomach, the withdrawal in large measure from the direct and concentrated action of the gastric juice of bacteria contained in ingested masses of food and large volumes of fluids, and the rapidity with which they may pass through the stomach into the intestine, we can understand how micro-organisms, even very susceptible to acids, may find frequent opportunity to enter the intestine. The normal stomach contains bacteria which find no difficulty in living there.

The secretions of mucous membranes in general afford conditions suitable for development to only a comparatively small number of bacteria, and they are distinctly injurious or even fatal to the life of many bacteria.

We have furthermore to consider that microorganisms find at the very gates of entrance living cells and fluids which destroy many of them, and even if they pass these gates they are likely to be arrested at the nearest lymphatic glands, which are probably especially adapted to overcome many species of bacteria.

The tendency of modern bacteriological investigations has been to assign much less importance than formerly to the air passages and lungs as the portals for entrance of infectious agents. Typhoid fever and Asiatic cholera, which were formerly referred to infection by the entrance of germs into the air passages, are now generally attributed to infection through the alimentary canal. Experiments seem to indicate that only such bacteria as are capable of multiplication in the lungs can produce infection through this organ, but we have no right on the basis of these experiments to draw conclusions as to a similar behavior of other classes of microorganisms; for example, the malarial plasmodium and the unknown infectious agents of the acute exanthemata. We must also be guarded in applying to human beings results obtained from forced inhalation or direct injection of large quantities of bacteria—as, for example, tubercle bacilli, pneumonia cocci, anthrax bacilli—into the air passages and lungs.

Infection through the inspired air, which will be subsequently considered. is not synonymous with infection through the air passages and lungs, as organisms which enter in other ways may grow or be carried down into the air passages, and those which have been inhaled may be swallowed.

Channels of Discharge.—The manner of transmission of an infectious disease is influenced, often decisively, by the way in which the infectious microorganism is discharged from the body.

It was once thought that the living body gets rid of invading microorganisms almost wholly by discharge through the kidneys, intestine, skin, or other emunctory channels, and the old doctrine of critical discharges and the use of eliminative methods of treatment were based upon a similar conception as to unknown morbific agents. We now know that the living cells and fluids of the body may possess or acquire germicidal and antitoxic powers which are far more powerful weapons of attack or defence against invading bacteria than are furnished by eliminative functions. Hence we are not surprised to learn that microorganisms are often disposed of in the interior of the body, and are then not discharged at all as living organisms.

The presence of infecting bacteria in the discharges and their situation depend, in the first place, upon the parts of the body occupied by the bacteria in question. Bacteria which themselves or by their products damage excretory organs or channels find the way opened for their elimination through these diseased parts. It has been stated, on the basis of experiments, that bacteria in the circulation are never discharged from the body through the healthy organs. Most pathogenic bacteria conform to this general law, but there are some which may appear in excretions without any demonstrable lesion of the organ through which they have passed. In general, however, our knowledge of the pathological anatomy of a disease and the distribution of the causal microorganisms enables us to predict in which excretions we are likely to find these microorganisms.

Thus the specific microorganisms are in the sputum in cases of pulmonary tuberculosis, pneumonia, diphtheria, influenza, rabies; in the intestinal discharges, sometimes in the vomit, in typhoid fever, Asiatic cholera, dysentery, intestinal anthrax, and tuberculosis; in the urinary or genital secretions in gonorrhoea, syphilis, genito-urinary tuberculosis, puerperal fever; in the discharges of wounds in tetanus, malignant oedema, traumatic infections; in or on the skin in cutaneous parasitic diseases, lupus, leprosy, erysipelas, typhus fever, smallpox, scarlet fever, measles, rubella. We have no information how the specific organisms are discharged in yellow fever, relapsing fever, dengue, and some other infections. The expired breath is free from microorganisms, except as they may have been mechanically detached in acts of speaking, coughing, sneezing, or hawking. The breath, therefore, is not the dangerous source of infection which it was once supposed to be. The milk may contain the specific bacteria in certain infections, particularly tuberculosis in cattle and those caused by pyogenic bacteria. A considerable number of bacteria are eliminated through the bile.

It will be observed that the typically contagious diseases, in the ordinary use of this term, are in large part those in which the specific organism is thrown off from the skin, whereas those diseases in which the infectious agent is discharged exclusively or chiefly by the intestine are for manifest reasons not likely to be directly conveyed from person to person, especially as it is chiefly these diseases which are contracted by swallowing the infectious material. In uncleanly and crowded quarters, however, direct transmission of Asiatic cholera and typhoid fever from the stools is not particularly uncommon.

In malaria we have no evidence that the plasmodium is ever discharged from the body in a living state, so that a malarial patient cannot under natural conditions infect either directly or indirectly another person or a locality. Malaria, however, can be artificially conveyed by injecting blood of a patient containing the plasmodium into a healthy person.

Sources and Ways of Infection.—The inferences to be drawn regarding the *infected individual* as an immediate source of infection to others are sufficiently evident from what has already been said concerning the modes of elimination and of reception of pathogenic organisms. The indirect dangers from this source are more conveniently considered in connection with the following external sources of infection, which as regards many diseases represent simply ways of transportation of infectious material from an infected individual to another individual. These media are so numerous and varied that only the more common and important ones can be here specified.

Formerly the *air* was considered the principal medium for the diffusion of epidemic diseases. The patient and his discharges were believed to give off emanations infecting the surrounding air. Even in ectogenous infections the air was regarded as the chief carrier of infection. Contagia transmissible through the air were described as volatile in distinction from fixed contagia.

The tendency now is to restrict within comparatively narrow, perhaps too narrow, limits the possibilities of infection through the air. The principal reasons adduced for assigning to the air a relatively unimportant rôle as a carrier of infection are the following: The demonstration of the frequency and efficacy of other methods of infection, particularly of contact infection; the evidence of absence of danger, or the relatively slight danger, of infection from the air in surgical operations and some infections when every other possible infectious source has been excluded; the observation that in laboratory experiments infected animals rarely infect others through the air; the experimental evidence that the air passages and lungs do not permit the invasion of many kinds of pathogenic bacteria into the body; the demonstration that living microorganisms are less abundant in the air than was formerly supposed; the infrequency with which pathogenic germs have been detected in the air; the immense volume of air causing rapid dilution of floating germs, taken in connection with the increasing importance attached to the number of bacteria as a causal factor; the proof that only desiccated bacteria can be conveyed with dust into the air, and that cholera spirilla are killed in a short time by complete drying, and other bacteria may be weakened in vitality and virulence by desiccation, to which often is to be added the injurious influence of sunlight and of oxygen.

These arguments justify the conclusion that the dangers from air infection were formerly very much exaggerated. It is evident, however, that some of the points mentioned, such as the failure to demonstrate many pathogenic germs in the air, and the application to most human diseases of the mode of spread of diseases inoculated into animals, are of little account, and also that neither singly nor collectively are these points pertinent to all infectious diseases. Most pathogenic bacteria can withstand drying long enough to permit their transportation by the air. Most cholera spirilla are killed within twenty-four hours by drying, but, according to Uffelmann, some may survive as long as this under conditions permitting their conveyance with dust, so that the possibility of the transmission of Asiatic cholera through the air cannot be positively denied, although it must be so exceptional as not to enter into consideration in explanation of epidemics of cholera. Pyogenic bacteria, and more especially the pyogenic cocci, have been repeatedly found in the air, particularly in hospitals. Tubercle bacilli have also, although very exceptionally, been detected in the air of rooms occupied by consumptives.

Certainly nobody supposes that gonorrhoea, syphilis, or rabies can be conveyed by the air, but it would be difficult to mention many other human infectious diseases in which the possibility of such conveyance has been absolutely disproven, although there are several which one would be reluctant to admit to this class. The evidence that the malarial germ is carried by the air seems conclusive. Infectious agents thrown off from the skin on dried epidermal scales, as in the contagious exanthemata, can doubtless be transported by the air, although, as would appear, in sufficient concentration to cause infection only for a short distance, which, however, may be increased by crowding of patients and lack of free ventilation.

It is believed by many that the principal, although not the sole, sources of tuberculous infection are the sputa of individuals affected with pulmonary tuberculosis. The tubercle bacilli are resistant, and may readily be transported by the air from the dust of dried material containing them.

The evidence is irresistible that typhoid fever may be conveyed by the drinking water and milk; but there is less evidence that it can also be transmitted by the air, although the possibility of this must be admitted.

The possibility of the infectious agents of pneumonia, diphtheria, and especially of influenza, being received through the air is generally conceded.

Even where we admit the possibility of air infection, however, probably in most diseases other modes of infection are more frequent and serious sources of danger; nevertheless, it would be wrong to overlook the possible dangers from the air, the more so as we are in regard to many infectious diseases in the dark as to the extent of these dangers.

The air may be a carrier of infection to other media, as to water, milk, food, clothing, etc., and to other portals of entrance than the respiratory, as to the skin, wounds, and the ailmentary tract. It has already been mentioned that the breath in expiration is not ordinarily a carrier of germs of any kind.

The bacilli of tetanus and of malignant oedema are common inhabitants of the *ground.* These bacilli, especially those of tetanus, are found also with great frequency in the faeces of herbivorous animals, such as horses and cattle, and appear to be more virulent in this situation than in the ground, probably because they can multiply and produce toxic substances better here than in the ground. Various pathogenic staphylococci and streptococci have been repeatedly discovered in the ground. The anthrax bacilli live in the ground in regions where this disease is endemic. The malarial germ has never been detected outside of the human body, and it is possible that it resides in some intermediary host.

The ground receives the discharges of all sorts of infectious diseases. Particularly are to be considered diseases in which the germs are contained in the sputum and intestinal discharges, such as tuberculosis, typhoid fever, and cholera. The tubercle bacilli can scarcely find conditions permitting multiplication in the soil, but it has been proven that they may preserve their vitality at least for many months in this situation. The cholera spirillum and the typhoid bacilli may more readily find such conditions for multiplication, but in general the ground with its low temperature, its varying degrees of moisture and dryness, its exposure to sunlight, its insufficient nutriment, and especially its vast number of competing saprophytes, is unfavorable for the growth of these or of most other infectious bacteria. Nevertheless—and this is the important point—these bacilli may survive a considerable time in the ground. Experimental tests have shown that the typhoid bacilli may live for at least three to five months in the soil, and the cholera spirilla, although usually in these experiments dying in a few days, may survive two or three weeks. These experiments as to the survival of typhoid and cholera bacteria in the ground do not reproduce all of the natural conditions with sufficient precision to justify definite deductions as to the possible duration of life of these organisms in or on the ground, and there is evidence from other sources that these bacilli may find conditions for longer life in this situation than that indicated by these experiments.

The principal ways in which pathogenic germs may be conveyed to us from the ground are through dust in the air, by surface drinking water, by certain articles of food, particularly salads, vegetables, and fruits by insects, and by direct or mediate contact. Microorganisms from the ground may be carried by the air or in various other ways to all sorts of objects with which we may come into contact. Street dust of cities has been shown to be very rich in bacteria of all kinds, and among these have been found virulent tubercle bacilli, pyogenic cocci, and tetanus bacilli.

Pettenkofer and his followers attribute to the soil peculiar and indeterminate properties and an exclusive rôle in the spread of typhoid fever and cholera, but we cannot discuss here the hypotheses upon which this doctrine rests.

Water as a carrier of infection comes into question in temperate climates almost exclusively as regards typhoid fever, cholera, dysentery, and diarrhoeal affections of indeterminate causation, although its agency cannot be positively excluded in some other infectious diseases. Notwithstanding the lingering opposition of a few hygienists, it may be considered as definitely settled that typhoid fever and cholera may be contracted from contaminated drinking water.

Bacilli presenting the essential features of the typhoid bacillus have been repeatedly demonstrated in water suspected of causing typhoid fever, although such bacilli have been more frequently missed under these circumstances. Nevertheless, the positive identification as typhoid bacilli of bacteria obtained from external sources is so extremely difficult—according to some, practically impossible—in consequence of the large number and wide distribution of pseudo-typhoid bacilli, that it is doubtful how far we can utilize these recorded observations in support of the origin of typhoid fever from drinking water.

Cholera spirilla have been found by Koch in the water of a tank in India, and during the epidemics of 1892-93 in Germany in the water of the Elbe at Hamburg, in a well in Altona, and at Nietleben in the Saale and conduit-water supplying the insane asylum where a severe cholera epidemic prevailed, and on the irrigation fields receiving the sewage from the asylum and discharging it again into the Saale at a point near the source of supply of drinking water. The spirillum was also found by C. Fraenkel and Lubarsch in water during the recent epidemic. The experiences of this epidemic have established upon an incontrovertible basis the long-disputed doctrine of the drinking water origin of cholera.

Many experiments have been made to determine the duration of life of cholera spirilla and typhoid bacilli in water, and with divergent results. Most of these show that in natural waters (unsterilized) the cholera spirilla not only do not multiply, but they die within two or three days, but there are several experiments which show that the cholera spirillum may survive a week. Koch found them still alive after eighteen days in a litre of water taken from a well in Altona and kept at a temperature of 3° to 5° C. The typhoid bacilli may live longer, but according to most experiments these too disappear within a few days up to two weeks from natural waters. Both may live for months in sterilized natural waters.

It is to be remembered that it is very difficult in these experiments to imitate all of the conditions of nature, and it may readily happen that these bacilli may find in some nook at the edge of a stream or well or on some floating objects or in a conduit better conditions for life, and even for multiplication, than would appear from laboratory experiments with water.

Water is rendered a source of infection mainly through contamination with the discharges of infected individuals. Hence any water where the possibility of such contamination exists is to be suspected as a source of supply for drinking purposes or household use. Subsoil water derived from the deep strata of the soil, even when the superficial layers of the latter are crowded with bacteria, is ordinarily practically free from bacteria, especially pathogenic species. If the proper care be taken in procuring it, water which has been thus filtered through the ground, is the safest source of supply. Unfortunately, the common way of procuring this water by dug wells exposes it frequently to most dangerous pollution, so that ordinary well water in many situations is a particularly dangerous source of infection.

There are various household uses of water, aside from drinking, through which it may convey infectious germs.

Experiments have shown that the susceptibility of animals to infection with the typhoid bacillus may be strikingly increased by injecting certain bacteria, and especially their products. Thus the products of *Streptococcus pyogenes, Bacillus coli communis,* and the common putrefactive bacillus, *Bacillus proteus vulgaris,* render rabbits and guinea pigs more susceptible to infection with the typhoid bacillus. These facts, as well as other observations, suggest that certain bacteria and products of decomposition in water, in food, and in the intestine may play an important rôle in predisposing to typhoid fever. Vaughan has found that waters suspected of causing typhoid fever are particularly likely to contain poison-producing bacteria. Contaminated water, therefore, may perhaps predispose those drinking it to typhoid fever independently of the presence in it of the typhoid bacillus.

There are various ways beside that through the agency of water by which the germs of typhoid fever and cholera may be conveyed. There is no occasion for adopting any exclusive theory as to the manner of communication of these diseases, of which cases are not uncommon in which the source of infection cannot be traced.

A careful study of the origin, spread, and distribution of epidemics of cholera or of typhoid fever will often indicate whether or not the epidemic is traceable to infection of the drinking water. It is especially the explosive outbursts of cholera which are attributable to infection of a general source of water supply. Here many persons in different parts of the locality supplied by the same water are simultaneously or in quick succession attacked. A second type of cholera epidemics is represented by those in which cases of the disease develop more slowly in scattered groups or foci, especially among the poor living in crowded and insanitary quarters. Here the infection is traceable to cholera patients, less frequently, however, to direct contact with them or their discharges than to infection from contaminated clothing, bedding, furniture, food, insects, etc. These two types of cholera epidemics often become combined or one may merge into the other.

Many bacteria preserve their vitality in *ice* for a long time—the typhoid bacillus, for example, for 103 days in Prudden's experiments. Hence ice should not be procured from water which is unfit for drinking purposes. Uffelmann and Renk have found that cholera spirilla do not survive in ice longer than six or seven days. Cold weather, however, does not prevent outbreaks of cholera, as several severe winter epidemics have occurred when the temperature was below the freezing point.

Unlike the external sources of infection thus far considered, many articles of *food* afford excellent nutritive media for the growth of a number of species of pathogenic bacteria, and this growth may occur without appreciable change in the appearance or taste of the food. The danger from infection from this source comes into consideration for uncooked or partly cooked food, and for food which, although it may have been thoroughly sterilized by heat, is allowed to stand a considerable time before it is used. Milk, in consequence of its extensive employment in an unsterilized state, its exposure to contamination, and the excellent nutritive conditions which it offers to many pathogenic bacteria, should be emphasized as especially liable to convey certain kinds of infection.

There is strong evidence that the germs of tuberculosis, typhoid fever, and cholera, and probable evidence that those of scarlet fever and diphtheria, may be conveyed by the milk. Tuberculosis is in some regions as prevalent among cows as among human beings, and tubercle bacilli may be in the milk not only when the udder is tuberculous, but when it is healthy, or at least when tuberculous nodules in it are so small as readily to escape detection. There is no evidence, however, that tuberculosis is conveyed by the milk of tuberculous women, although if, as rarely happens, the mammary gland be tuberculous, such an occurrence would not be improbable.

Here may be mentioned the not uncommon instances of poisoning, often of a large number of people (Massenvergiftung), from the ingestion of decomposing or altered fish, mussels, oysters, sausage, canned meats, ham, milk, cheese, and ice cream. These are due to intoxication, and chiefly to the class of bacterial poisons called ptomaïns produced in the early stages of certain kinds of decomposition. In certain cases of poisoning with milk, cheese, and ice cream Vaughan has demonstrated a toxic ptomaïn which he calls tyrotoxicon. It is not improbable that the bacteria producing these poisons may continue their activity in the alimentary canal, but there is no evidence that these bacteria, unaccompanied by their toxic products, are harmful when ingested by human beings. It is uncertain whether any of these bacteria are capable of causing genuine infection, although in some fatal cases of these intoxications bacteria have been found in the organs of the body. The best studied of these invading bacteria is *Bacillus enteritidis* of Gärtner, to which, in fact, he attributes the disease in which it was found.

Insects, especially flies, may assume the sensational rôle of messengers of infection. They may carry infectious agents not only to persons, but also to articles of food and other objects. It has been demonstrated that the bacilli of tuberculosis, typhoid fever, Asiatic cholera, anthrax, and *Staphylococcus aureus* may pass unchanged through the alimentary canal of flies into the excrement. Sawtschenko found living cholera spirilla in the excrement of flies fed three and four days previously with cholera cultures or with cholera excreta. Mosquitoes may possibly be capable of conveying infection by inoculation.

Various *other objects,* especially such as have been in contact with or proximity to infected persons, as clothing, bedding, furniture, household utensils, animals, the hands, dressings, and instruments of physicians, surgeons, and midwives, and other things too numerous to specify, are manifest and common carriers of infection. Substances which contain contagia are often called *fomites.*

With all sources of infection it is actual personal *contact* with the infected objects which involves the chief dangers.

In this connection a few words may be said concerning infection with *pathogenic bacteria found in healthy persons* more or less frequently, some so constantly that they belong to the normal bacterial flora of the body. The unhappily chosen term "auto-infection" is sometimes given to some of these infections. The bacteria which are most commonly concerned are *Micrococcus lanceolatus,* pyogenic cocci, especially streptococci, and *Bacillus coli communis,* but occasionally other pathogenic species, are represented.

Members of this class of bacteria are the ones especially concerned in *secondary* and *mixed infections.* Infectious diseases which cause lesions of mucous membranes, such as tuberculosis, diphtheria, scarlet fever, typhoid fever, open the way to the invasion of these bacteria both by these lesions and by reducing the resistance of the body. The secondary streptococcus infections add to the gravity especially of tuberculosis, diphtheria, and scarlet fever, in which they are very common. In healthy persons these pathogenic bacteria are not likely to do any harm by their presence in the alimentary canal or on other exposed surfaces. These bacteria may, however, under conditions not at present understood, acquire unwonted virulence within the body.

The presence in the mouths of 15 to 20 per cent of human beings of *Micrococcus lanceolatus* in a sufficiently virulent state to kill rabbits, and still more frequently in a less virulent condition, bears upon the causation of croupous pneumonia, and doubtless in the sense that predisposing conditions are necessary in many persons to permit the infection of the lungs with this organism.

The occasional existence in the mouth of nonvirulent bacilli, apparently otherwise identical with the Klebs-Löffler bacillus of diphtheria, is a significant fact, but not at present clear in its interpretation in relation to the causation of this disease.

Germinal and Intra-Uterine Infection.—The embryo may be infected through an infectious agent in or attached to the ovum or in the semen. This is called *germinal* infection. The best established instance of this is found in an epizootic disease, called pebrine, destructive to silkworms and caused by minute parasitic corpuscles regarded by Balbiani as sporozoa. These peculiar parasites occur both in the ovum and in the semen, and it has been proven by Pasteur and all who have investigated the subject that ova containing them are capable of regular development and generate silkworms infected with the same microorganisms.

The evidence seems conclusive that congenital syphilis is usually due to germinal infection, and that it may spring from either a syphilitic father or a syphilitic mother.

The possibility that the bacilli of tuberculosis and of leprosy may be transmitted by germinal infection is strongly urged by some of those who consider inheritance of these germs an important factor in causation. There is some evidence that germinal infection of fowls with tubercle bacilli occurs, but no satisfactory evidence of the same mode of infection in mammalian tuberculosis. The bacilli of avian tuberculosis are different from those of human tuberculosis.

In *intra-uterine* or *placental* infection of the foetus the infectious agents reach the embryo in most, if not all, cases through the blood-circulation by way of the placenta. There are possibilities of infection through the lymph channels of the umbilical cord, through the membranes and amniotic fluid, and through the Fallopian tube and wall of the uterus.

The placenta is a perfect physiological filter without direct communication between the blood of the mother and that of the foetus. Inanimate particles do not pass through the intact placenta into the foetal vessels. Pathogenic organisms, however, may be capable of injuring the placental tissue and of growing through unruptured vascular walls.

Both experiments upon animals and observations on human beings have shown conclusively that infectious agents may pass from mother to foetus through the placenta. The bacilli of chicken cholera and of symptomatic anthrax and pyogenic cocci frequently make this passage, demonstrating that some microorganisms are better adapted than others to break through the placental barrier. Anthrax bacilli may pass from mother to foetus, more frequently in some species of animals than in others, but generally in such small number as to require careful search for their detection.

In human beings foetal infection through the placenta has been observed in smallpox, measles, scarlatina, relapsing fever, syphilis (?), croupous pneumonia, typhoid fever, affections caused by pyogenic cocci, anthrax, and, it is claimed, in one case of Asiatic cholera. In every one of these diseases such transmission, so far as demonstrated, is exceptional, more so in some than in others.

Especial importance attaches to the question of inheritance of tubercle bacilli. Baumgarten advocates vigorously the doctrine that heredity as a causal factor in tuberculosis is mainly inheritance of the bacilli, and not, as is usually believed, inheritance of the soil or predisposition. Baumgarten's doctrine, received at first with great opposition, is gaining adherents, although the weight of authority is still decidedly against it. Instances of congenital tuberculosis, proven beyond doubt, are few; still there are such in both human beings and animals. Johne's first conclusive demonstration of tuberculosis in a foetal calf was regarded at the time as a unique curiosity. It now appears, however, that similar cases are not very uncommon. Developed tuberculosis of the human foetus is extremely rare. Baumgarten found in a stillborn infant a tuberculous caseous nodule in the upper cervical vertebrae. More common, but still infrequent, are cases of more or less advanced tuberculosis in infants dying within the first few days after birth. These cases must certainly be regarded as congenital, and probably also the same interpretation must be given to at least some of the cases dying within the first few weeks of extra-uterine life. The frequency of tuberculosis increases very rapidly with each succeeding week after birth, until during the second half of the first year and during the second year of life fatal tuberculosis is very common, and then diminishes in frequency until after puberty.

Birch-Hirschfield detected in a seven months' human foetus and in the placenta, removed by Caesarean section from a mother affected with acute miliary tuberculosis, tubercle bacilli both by microscopical examination and by inoculation of guinea pigs. There were no tuberculous lesions in the foetus. A similar demonstration has been made by Armanni and by Aviragnet. A larger number of negative results in the same class of cases have been reported.

Until Gärtner's important publication in 1893 comparatively few positive, and many negative, results had been obtained by experiments on animals, both as regards developed foetal tuberculosis and the presence of tubercle bacilli in the foetus without tuberculous lesions. Bacilli have been found far more frequently than tubercles in these experimental cases. Gärtner finds that the transmission of tubercle bacilli from mother to foetus is not at all uncommon in tuberculous mice, canary birds, and rabbits, the last inoculated into the circulation. By inoculating before incubation hen's eggs with bacilli of fowl tuberculosis, Maffucci found that six of eight chicks from the hatched eggs died from tuberculosis in twenty days to four and a half months after birth, precaution being taken to avoid accidental infection. The bacilli remained apparently quiescent in the embryo until birth. These interesting results are not interpreted by Maffucci as due, strickly speaking, to germinal infection, but rather to infection through the area vasculosa, and hence as analogous to placental infection. The possibility of the passage of tubercle bacilli from the mother into the ovum has been demonstrated by Gärtner, who obtained nine eggs from twelve canary birds which had been inoculated in the abdominal cavity with tubercle bacilli. Two of these eggs were proven by inoculation of guinea pigs to contain tubercle bacilli.

The infrequency of tuberculosis in the human foetus and the newborn is not a conclusive argument against frequency of placental infection of the foetus with tubercle bacilli, when one considers the chronic development of the disease, the possible relative insusceptibility of the embryo, the small number of bacilli likely to penetrate the foetus, and the probability that the conditions are more favorable for placental transmission of tubercle bacilli during the latter part of pregnancy, and especially during parturition, than at an earlier period. We have evidence that tuberculous processes with living and virulent tubercle bacilli may remain latent in the body a long time; and this doctrine of prolonged latent tuberculosis is an essential part of the hypothesis that tuberculosis is often due to bacillar heritage. But notwithstanding the increasing plausibility of the theory of frequent bacillar heritage, many arguments, which cannot be considered here, can be urged in opposition, and the final settlement of the question involves great difficulties not likely to be soon overcome.

We have little information as to the conditions which occasionally permit the passage of microorganisms from mother to foetus. These conditions are sometimes to be found in lesions of the placenta, either preexisting or caused by the microorganisms themselves or their products. Circulatory disturbances, particularly haemorrhages, defects in the epithelium of the chorionic villi, and areas of necrosis, have been observed with more or less frequency by different observers in cases of foetal infection, but often no lesion could be detected. During parturition the chances of placental transmission of microorganisms would seem to be increased, both by circumstances favoring the passage of organisms into the blood of the mother and by increased blood pressure and ruptures in the placenta.

It is to be noted that, the portal of entry to the foetus being usually the umbilical vein, microorganisms would be carried first to the liver and the right side of the heart. Corresponding to this, we find that the organisms are generally most abundant in the liver, and that hepatic lesions are particularly common in congenital infections. Possibly the greater frequency of right-sided endocarditis in congenital heart disease is to be explained in the same way. Pyogenic cocci, which are the bacteria most often associated with endocarditis, break through the placental barrier with comparative ease. Infantile tuberculosis is not, however, nearly so frequent in the liver as in the lymphatic glands, lungs, bones, and joints—a fact which has been urged against congenital transmission of the bacilli. On the other hand, it is especially the frequency of cases of tuberculosis in lymphatic glands, joints, and meninges, without tuberculous lesions at any of the portals of entry, which are considered to support the theory of congenital infection.

With some diseases the foetus when infected reacts essentially in the same way as the mother, the affection being sometimes more severe, sometimes less so, than in the mother. With some bacteria, however, the characteristic lesions of the disease have not been reproduced in the foetus, and the conditions appear to be unfavorable for multiplication of the invading organisms. This, however, is not certain, as it may be that the bacilli in such cases have been too short a time in the foetus to develop and cause lesions. No instance, known to the writer, has been observed in the foetus of fully-developed anthrax, of croupous pneumonia caused by *Micrococcus lanceolatus,* or of intestinal lesions by the typhoid bacillus, although in several recorded instances these bacteria have unquestionably invaded the foetus from the mother. The characteristic lesions have, however, been found so soon after birth as to indicate positively congenital infection. It is interesting to note that the pneumonia cocci and the typhoid bacilli may produce septicaemia without local lesions in the foetus and congenitally infected newborn. Maffucci found that some pathogenic bacteria inoculated into hen's eggs survived, but did not usually develop until after the birth of the chick. This was true of the bacilli of chicken cholera, to which fowls are very susceptible. This apparent insusceptibility of the embryo to some species of pathogenic bacteria is the more remarkable when we consider that young animals are generally, although not invariably, more susceptible than adults.

Toxic substances, often causing foetal death, may pass much more readily from mother to foetus than the microorganisms producing the poisons.

Predisposition.—A consideration of the general etiology of infections which leaves out of sight important causal factors relating to the individual exposed to infection would be extremely misleading and one-sided. It is convenient to consider these factors under the comprehensive and heavily burdened term "predisposition." Unfortunately, while conceding the important, often decisive, rôle of individual predisposition for many infectious diseases, we can rarely define the precise nature of the predisposing conditions. We often have to resort to such mere phrases as that the tissues of one person offer better conditions for the growth of a given microorganism than do those of another.

Instances abound of differences of susceptibility to disease between different species or races of animals, but this racial predisposition is to be distinguished from predisposition as applied to individuals of the same species.

Individual predisposition is not a factor of equal importance for all infectious diseases. It comes into consideration chiefly for those diseases to which the species or race is not in the highest degree susceptible, and for infectious microorganisms of weakened virulence. There are certain diseases, such as smallpox and measles, to which human beings (unvaccinated) are so susceptible that predisposition becomes a factor of minor importance. There are other diseases, such as tuberculosis and croupous pneumonia, in which predisposition controls the etiology of the disease.

The fact that some individuals are attacked, and others, apparently equally exposed to the danger of infection, escape, is not always due to any especial predisposition on the part of the former. It may be that the germs hit the one and miss the other, and we would have no more right to say that the former are especially predisposed than to say that those who fall in battle are predisposed to bullets and those who escape are bullet proof. The truth of this is shown by the fact that those who at first escape may be attacked upon subsequent exposure.

The degree of susceptibility to a pathogenic microorganism influences not only the capacity to acquire the disease, but also the course, severity, and character of the disease. An infectious disease is generally less severe, and, if fatal, kills after longer duration, when there is slight susceptibility. In animals especially, and to some extent in human beings, many species of infectious bacteria remain localized, causing more or less extensive inflammations, in the relatively insusceptible, and the same organisms invade and multiply in the blood, causing general septicaemia, in the highly susceptible. These differences are well exemplified by the differences in the behavior of *Micrococcus lanceolatus*. Differences in the course and character of tuberculosis may be explained in large measure by differences in susceptibility to the tubercle bacillus; whether also to differences in virulence of the tubercle bacillus is uncertain, but not improbable.

It would appear that varying individual susceptibility plays a more important part in the etiology of most infectious diseases in human beings than it does in animals used for experimentation, although there are many instances of its importance even in the latter. Rats, which are relatively insusceptible to anthrax, have been used to test the influences of predisposing causes of disease.

Individual predisposition may be either local or general; that is, may be due to conditions affecting either a part or the whole of the body.

First in importance among the local predisposing causes are those affecting the portals of entrance of infectious organisms. The situation and the normal defences of these portals have already been considered. There are many obvious ways in which these defences may be weakened or overthrown. Here may be specified wounds, ulcers, inflammations, necroses, haemorrhages, presence of foreign bodies, alterations in secretions of mucous membranes, trophic disturbances due to impaired nerve influences. Gastric disturbances, which reduce the acidity and weaken the peristalsis of the stomach, increase the susceptibility to cholera. Many of these defects not only open a passage for microorganisms, but afford conditions favorable for their development by lessening the vital resistance of the part. A great deal depends upon the invader getting a foothold where it can multiply, if only a little, and produce toxic products which often constitute its real weapons of attack. Besides the grosser lesions mentioned, there may be less evident congenital or acquired abnormalities. Local predisposing conditions are most highly operative when, as often happens, they are associated with general predisposition. Furthermore, the general causes often act by inducing local changes. Finally, when all has been said, we must admit that infectious germs may enter the body, we know not how or where.

After the infectious agents have entered the body they may find local conditions favoring their lodgment and development. Some microorganisms will not grow in the blood; many show a preference for certain organs and tissues. Often we have no more satisfactory explanation to offer for the various localizations of infectious diseases than to say that some organs and tissues offer more favorable conditions for the life of certain organisms than do others. Sometimes we can explain the localization by the manner of reception of the virus, the vascular relations of the part to infected areas, the size and number of the capillaries, and the readiness with which foreign particles are filtered out.

Injury, inflammation, and other preexisting disease of a part are important and frequent conditions favoring the lodgment and growth of microorganisms. For example, bacteria do not readily become attached to the smooth surface of the heart valves, but they may adhere and develop when the valves have been torn or have been roughened by disease. The predisposition of injured joints and bones to the settlement of tubercle bacilli and of pyogenic bacteria is well established, both by clinical and experimental observations. Without some such *locus minoris resistentiae* some pathogenic bacteria may enter the circulation and be destroyed without doing any appreciable harm. The anaemic and dry condition of the lung induced by pulmonary stenosis favors the development of pulmonary tuberculosis. The hyperaemic and moist condition associated with mitral regurgitation is comparatively unfavorable to such development, although by no means excluding this disease. The inhalation of foreign particles, as in the case of miners and stone-cutters, predisposes to tubercular disease of the lungs. Bronchial catarrhs, bronchopneumonias, especially those accompanying measles, whooping cough, and some other diseases, are also local predisposing causes of tuberculosis; but here it is not easy to separate local from general predisposition.

Organs and parts of the body may inherit special vulnerability to certain infections.

Susceptibility to certain infectious diseases may be manifest in races and families. The negro race is less susceptible to yellow fever than the white. Algerian sheep are in large measure insusceptible to anthrax, which is very fatal to other sheep. It would seem, as regards some infectious diseases, as if by a process of natural selection relatively insusceptible races were developed. The frightful mortality of measles freshly introduced among uncivilized peoples, as in the Sandwich and Fiji Islands, is usually cited as a case in point. Black rats are more resistant than gray, and gray rats more resistant than white, to anthrax.

The factors concerned in general predisposition are for the most part less tangible than those of local predisposition. Here also we distinguish congenital and acquired predisposition. Age is a predisposing condition. Certain infectious diseases are most common in infancy, others in adolescence or in maturity or in old age. As a rule, young animals are more susceptible to infections than old ones. There is, however, a special insusceptibility of sucklings during the first months of life to certain infectious diseases, such as scarlet fever, measles, mumps. It is questionable whether there is any difference in predisposition to infection between males and females, except as regards infections directly related to sexual functions.

Impaired vitality and nutrition of the body may predispose to certain infections. More or less plausible predisposing causes operating in this way are bad and insufficient food, overwork, depressing emotions, exposure to extremes of heat or cold, overcrowding, bad air, and, in general insanitary surroundings. There are experiments on animals showing the effect of food, hunger, overwork, anaemia, and abnormal temperature in increasing susceptibility. Feser, Hankin, and Müller found that rats fed on bread are more susceptible to anthrax than those fed on meat. Canalis and Morpurgo, and Sacchi rendered relatively insusceptible pigeons highly susceptible to anthrax by hunger. Charrin and Roger increased the susceptibility of rats to anthrax and to symptomatic anthrax (Rauschbrand) by making the animals run a treadwheel several hours a day. Anaemia induced by bleeding has been shown to increase the susceptibility of certain animals to various microorganisms, including the anthrax bacillus, the pneumo-bacillus of Friedländer, and *Staphylococcus pyogenes aureus.* Pasteur made hens, which are normally insusceptible to anthrax, susceptible, by artificial refrigeration. Petruschky and Gibier rendered naturally immune frogs susceptible to anthrax by elevating their temperature. Prolonged abstinence from water has been found by Pernice and Alessi to render relatively insusceptible animals more susceptible to anthrax. It is of course to be understood that these various and suggestive results may be explained in different ways, and that they relate only to certain microorganisms and certain animals, and cannot be directly applied to conditions in human beings.

On the other hand, as regards some diseases—for example, typhoid fever—well-nourished, robust young men are at least as susceptible as those with impaired nutrition.

Diabetes mellitus markedly increases susceptibility to infections, particularly with tubercle bacilli and pyogenic micrococci. By feeding white mice with phloridzin, which produces glycosuria, Leo rendered these animals highly susceptible to glanders, from which they are normally immune. The influence of certain infectious diseases in favoring secondary and mixed infections has already been referred to.

Various chemical substances introduced into the body, such as lactic acid in the case of symptomatic anthrax and tetanus, and particularly certain bacterial products, may increase susceptibility to some infectious organisms. Substances, such as pyridin, which destroy the corpuscles and break up certain albuminous constituents of the blood, may lower the resistance to certain infections. Certain fermentative and putrefactive processes in the stomach and intestine may by auto-intoxication increase susceptibility to infection.

Narcosis may impair resistance to some infections. Klein and Coxwell made frogs and rats highly susceptible to anthrax by narcosis with ether and chloroform, and similar results have been obtained with curare, alcohol, chloral, morphine, and upon other animals and with other diseases.

Climate, altitude, seasons, atmospheric humidity, are believed to influence general predisposition, especially toward tuberculosis and tropical diseases.

By repeated exposure to certain infectious agents resistance seems to be increased. Pathological anatomists are, as a rule, less likely to become infected by post mortem wounds than are those who rarely make autopsies.

Differences in individual susceptibility are well illustrated by the fact that some pathological anatomists are very prone to develop necrophilic tubercles (Leichen-tuberkel), whereas others rarely or never acquire them.

For a while after the subsidence of an epidemic insusceptibility of the survivors, including those who have manifested no symptoms of the disease, is often apparent.

Finally, in many cases we are unable to discover any cause for existing predisposition, and can only say that such susceptible persons by inheritance or by acquirement possess cells and fluids unable to cope with invading microorganisms.

Many of the predisposing factors which we have considered weaken or abolish this power of the living cells to overcome bacteria. We shall consider under the heading of Immunity more particularly this vital power which distinguishes an immune from a susceptible individual.

TOXIC PRODUCTS OF BACTERIA

The bacteriological study of infectious diseases has led to the conviction that the phenomena of these diseases are due in great part to poisonous substances formed by the causal microorganisms. Even before the discovery of the specific microorganisms purely clinical observation had suggested a similar conclusion in the case of many of these diseases. Our positive information on this subject relates at present chiefly to those diseases caused by bacteria, although we have evidence of the formation of toxic substances in some of the infectious diseases not known to be produced by bacteria.

If we compare the distribution of the bacteria in cases of infectious disease with the character of the symptoms and situation of the lesions, we shall find that we are often unable to explain the latter on any other supposition than that they are produced by absorbed toxic products. The most conclusive proof of this is furnished by those diseases in which the bacteria develop only or chiefly locally, sometimes to a very limited extent, at and around the point of entrance, and nevertheless profound constitutional disturbance, perhaps with marked lesions elsewhere, is produced. The best examples of such toxic diseases are diphtheria and tetanus. Next in order are malignant oedema and symptomatic anthrax in animals. Probably Asiatic cholera belongs also to this toxic group.

At the other extreme are the preeminently infectious, in distinction from the toxic, diseases. Here we find the blood and organs swarming with bacteria. Bacteriologists are accustomed to restrict the name septicaemia to diseases characterized by the presence of many bacteria in the blood—a usage not altogether in accordance with the common and traditional sense of this word. We must look to the lower animals for conspicuous examples of these septicaemias in the bacteriological sense, although they are not unknown in human beings. Examples are anthrax, chicken cholera, mouse septicaemia, erysipelas, pneumococcus septicaemia. Even here we have evidence of production of toxic substances, for the bacteria causing these affections develop at first locally, disappearing for a while from the circulating blood if they have been at first directly introduced into it, and often they do not make their appearance in any considerable number in the circulation until a few hours before death and after fever and other constitutional symptoms have set in. Nevertheless, as might naturally be expected, we do not find such concentrated and potent poisons in these bacterial septicaemias as in the more purely toxic affections.

Between the extremes mentioned we have all grades of transition, as, for example, local infections without marked constitutional disturbance, local infections with varying, sometimes much, sometimes little, general disturbance, and infections with more or less wide distribution of the bacteria in different organs, with preference for certain organs according to the disease, but without much or any development of the organisms in the blood. Typhoid fever is an example of the last class, which is often characterized by decided evidences of toxaemia.

Many pathogenic bacteria produce specific poisons not only in the animal body, but, fortunately for purposes of investigation, also in artificial cultures, from which they can be separated and studied as to their chemical and biological properties. Evidence of this is that we can poison animals with sterilized cultures of pathogenic bacteria, and sometimes can reproduce in this way many of the characteristic symptoms and lesions of the disease.

Search was naturally first directed to substances of definite chemical composition, more particularly to crystallizable substances which offer a guarantee of chemical purity. This search was rewarded by the discovery of a large number of crystallizable alkaloidal substances called by Selmi ptomaïns. The larger number of these ptomaïns have been obtained from putrefying substances containing a mixture of undetermined bacteria. Some are poisonous, such as hydrocollidin, mydalein, neurin, cholin, muscarin, gadinin, methylguanidin, peptotoxin, tyrotoxicon, in slight degree cadaverin and putrescin; but many are innocuous. Mention has already been made of this class of bacterial poisons in considering poisoning from decomposing food.

We owe especially to Brieger the study of the ptomaïns formed by pure cultures of pathogenic bacteria. From cultures of the typhoid bacillus he obtained a poisonous ptomaïn called typhotoxin; from cultures of the cholera bacillus, which is an energetic splitter of organic substances, he separated no less than six ptomaïns, some poisonous; in impure tetanus culture he found four ptomaïns—tetanin, tetanotoxin, spasmotoxin, and an unnamed base. He was unable to discover a ptomaïn in cultures of *Staphylococcus aureus* or *Streptococcus pyogenes,* but Leber obtained from the former a crystallizable alkaloid called phlogosin, capable of causing suppuration. This substance has not since been found. Pyocyanin is an alkaloid produced by *Bacillus pyocyaneus,* found in blue pus, and a pathogenic organism of much interest. Pyocyanin and its derivatives are the coloring principles in the cultures of this organism and in blue pus.

No poisonous ptomaïns have been found in cultures of the anthrax bacillus in ordinary media, but Hoffa obtained a poisonous base from cultures on meat, and Martin has separated a poisonous alkaloid from anthrax cultures containing alkali albumin and from the bodies of animals and a man dead of anthrax.

The four ptomaïns obtained by Brieger from impure tetanus cultures produced spasms when injected into animals, and this result seemed to render this line of investigation promising.

It was found, however, that the toxic ptomaïns in general do not reproduce in animals the more characteristic symptoms of infectious diseases, and that they are absent from cultures of some highly toxic bacteria—for example, *Bacillus diphtheriae.* On this account, and more especially on account of the discovery of another class of substances which have been called toxalbumins or toxic proteids, and of which some do reproduce the characteristic symptoms, medical interest has waned as regards bacterial ptomaïns.

Doubtless the injurious effects of pathogenic bacteria are attributable in part to ptomaïns and other definite chemical substances of a poisonous nature, but these substances cannot claim anything like the importance and interest which attach to certain amorphous toxic products of bacteria which can be only very imperfectly defined chemically at the present time. It is not improbable that some of the poisonous ptomaïns are themselves derived from these so-called toxalbumins. The name "toxins" was given by Brieger to poisonous ptomaïns, but it is now often applied also to the toxic proteids, which may be designated "amorphous toxins" in distinction from the "crystallizable toxins," or poisonous ptomaïns.

We owe to Weir Mitchell and Reichert pioneer work in the study of toxic proteids. They found that snake venom owes its toxic properties to albuminous constituents. Various toxalbumins, such as abrin, ricin, robin, are produced by the higher plants. The chief impulse to the study of bacterial toxalbumins came from the researches of Roux and Yersin in 1888 on the nature of the poison produced by the diphtheria bacillus, and those of Brieger and Fraenkel in 1890 on the poisons of diphtheria, tetanus and other infectious diseases.

There is evidence that some of the so-called toxalbumins, notably the poison of tetanus, are not of an albuminous nature, and in fact there is no stringent proof that most of the remarkable substances which have been grouped under the toxalbumins are albumins. These amorphous toxic substances can often be precipitated with albuminous substances contained in cultures or the body fluids. It has been considered an open question as to whether the amorphous toxins thus precipitated are really of an albuminous nature or not. Brieger and Cohn have succeeded in separating the tetanus poison almost entirely from admixture with albuminous material, and as this poison has been more thoroughly studied than any other bacterial poison, and is a most typical member of the group of so-called toxalbumins, we may expect that the other members of the group will be separated from albuminous substances. In speaking of these amorphous toxins, therefore, as toxalbumins or toxic proteids, it is to be understood that this designation is only a provisional one in the absence of a better name, and even with our present imperfect knowledge is open to objection. Even if it be assumed that they are really proteids, of course this does not afford an insight into their chemical constitution. Chemically we cannot distinguish the toxic proteids from other known proteids, any more than we can define chemically the difference between living and dead albumin.

It would appear that several kinds of proteids are represented in this toxic group, such as globulins, nucleo-albumins, peptones, and albumoses. Some of the most typical are thought by some writers to be ferments or enzymes, to which in action they bear much resemblance. There is, however, little agreement of opinion as to their chemical position. It is by their biological, rather than their known chemical, properties that we are enabled to recognize and distinguish various toxic amorphous products of bacteria.

A distinction, which, however, is not of a fundamental nature, is made between two classes of amorphous toxic bacterial products: (1) those which readily appear in solution of liquid cultures and in the fluids of the animal body, not being firmly bound by the protoplasm of the living bacterial cells; (2) those which are incorporated in the substance of the bacterial cells and are set free, especially from degenerated or dead bacteria.

It is more especially the readily soluble amorphous toxins, of which typical representatives are the poisons of tetanus and diphtheria, which in contrast to the so-called bacterioproteins of the second class have been considered to be metabolic products formed by the action of bacteria directly from the proteid material in cultures or in the animal body. At least some of these toxins may, however, be formed by bacteria growing in culture media free from any trace of proteid material, so that it is probable that they are elaborated within the bodies of the bacteria and are set free in the manner of secretions, as we believe the toxic albumins of snake venom to be formed in the epithelial cells of the poison glands.

Buchner, to whom we owe the name "bacterioprotein," obtained from the bodies of a variety of bacteria proteid substances with common properties, being characterized especially by solubility in alkalies, resistance to boiling temperature, attraction of leucocytes, as manifested by leucocytosis and suppuration (positive chemotaxis), and pyrogenic power. Aside from these resistant bacterioproteins, common to many bacteria, some bacteria—for example, the cholera bacillus—may form unstable amorphous toxins, characterized by specific poisonous properties and intimately incorporated with the bacterial cells.

There is evidence that toxic substances formed by bacteria are sometimes quickly broken up into other, often harmless, substances by the bacteria or their products, and in this case their demonstration may become a matter of great difficulty. These toxic products constitute the chief weapons of attack of many pathogenic bacteria. These weapons are needed much more in the animal body than in cultures. There is evidence that these poisons may be discharged more readily under conditions attending the invasion of the body by bacteria, where they are needed by the invaders, than in cultures where they are not needed. Moreover, we know that some pathogenic bacteria may form toxic products in some culture media, and not at all or but little in other media, and also that some may produce poisons in the animal body and but little in artificial cultures. Hence, we are not to suppose that because we are unable to demonstrate specific poisons in certain culture media, such poisons or different poisons may not be formed in other culture media, and more especially under the divergent and more complicated conditions existing in the living body.

In fixing our attention upon the poisonous chemical products of bacteria we must not lose sight of the fact that these are the results of vital activities. It is only in a very few almost purely toxic affections like tetanus that the actual presence and multiplication of the bacteria in the body are not needed in order that the poison may produce the essential features of the disease. In the case of most infectious diseases we can no more dissociate the actual presence, multiplication, and specific vital activities of the bacteria within the body from the disease than we can substitute any chemical substances for the actual presence and growth of the yeast fungi in the production of alcohol from sugar. We cannot resolve bacteriology into toxicology.

Only those amorphous toxins which belong to the more important diseases of human beings can be considered in this article. The best studied are those of tetanus and diphtheria.

The specific tetanus poison has been demonstrated both in pure cultures of the tetanus bacillus and in the bodies of animals and of human beings dead of tetanus. Inoculated into susceptible animals—and there are very few not susceptible—it produces all of the symptoms of tetanus. The disease produced in our ordinary laboratory experiments by inoculating tetanus cultures is almost entirely an intoxication, not an infection. According to Kitasato, the poison is destroyed in five minutes at 65° C., in twenty minutes at 60° C., in one and a half hours at 55° C.; it stands drying at ordinary temperatures; is not injured by dilution with water or bouillon; is sensitive to acids and alkalies. Kitasato obtained liquid cultures of such virulence that 0.00001 c. c. of the germ-free filtrate, corresponding to 0.00023 mgm. of the dried substance from the filtrate, sufficed to kill a mouse with tetanus. Of course only a part of the dried substance is the real poison. So sensitive is the tetanus poison to chemical reagents that Kitasato was unable to find any means of precipitating the poison without considerable loss of toxicity. He therefore expressed ignorance as to the real nature of the poison.

Brieger and Cohn have, however, succeeded in obtaining the poison in a more concentrated and purified form from veal bouillon cultures of the tetanus bacillus. The poison is first precipitated from the germ-free filtrate of the culture by supersaturation with ammonium sulphate. The precipitate which rises to the surface of the fluid contains the poison. This is collected and dissolved in water. From this solution the albuminous substances are removed by careful addition of basic lead acetate with a trace of ammonia, the poison remaining in solution. Peptones and salts are eliminated by dialysis of the filtrate with running water. The dialyzed liquid is then evaporated in a vacuum at a temperature of 20° to 22° C., by which process volatile substances are removed and the poison is obtained in a concentrated form as yellowish, transparent flakes, readily soluble in water, odorless, with only a small amount of ash and without response to Millon's or the xanthoproteic reactions for albumins. It is free from phosphorus, and contains only an imponderable trace of sulphur, due probably to contamination with some other substance. It is precipitated by ammonium sulphate, but not by sodium chloride, sodium sulphate, magnesium sulphate, boiling and other characteristic precipitants of albumins. Unlike the diphtheria poison, it is not dragged down by calcium phosphate. Brieger and Cohn conclude that the tetanus poison is not an albuminous substance in the ordinary sense. As to its chemical nature nothing is definitely known. It is sensitive to chemical and physical agents, being injuriously acted upon by acids and alkalies, sulphuretted hydrogen, and high temperatures. Even when kept free from exposure to light air, and moisture it gradually loses its strength. In the dried state it is not destroyed by an absolute alcohol, chloroform, acetone, and anhydrous ether. Temperatures exceeding 70° C. completely destroy the dry poison.

The poison, as thus obtained, although from cultures of less primary virulence than those secured by Kitasato, is of almost incredible potency. As 0.000,000,05 grm. of the purified substance killed a mouse of 15 grm. weight, 0.23 milligram would represent the fatal dose for a human being weighing 70 kilo. When it is considered that the minimal fatal dose of atrophine for an adult is 130 milligrams and of strychine is 30 to 100 milligrams, some conception of the terrible energy of this bacterial weapon can be obtained. It is furthermore to be considered that the substance separated by Brieger and Cohn is far removed from absolute chemical purity.

The diphtheria poison has been demonstrated both in cultures and in animals and human beings dead of diphtheria. It has not been separated from admixture with albuminous substances, and is generally regarded as a toxic albumin, although for reasons already stated the propriety of this designation is open to question. As shown by Ouchinsky, the poison, although in a weakened form, may be produced by the growth of the diphtheria bacillus in media free from proteid constituents. The poison thus formed is believed by Ouchinsky to be of an albuminoid nature, as it was contained in substances responding to the reactions for proteids.

The quantity of the poison formed in cultures depends upon the composition, reaction, temperature and age of the culture and the virulence of the bacillus. The poison is most abundant in cultures four to eight weeks old at body temperature. It is obtained in approximate purity by filtering the culture through a Chamberland filter, partly evaporating the filtrate in vacuo at 30°C., and precipitating with alcohol. It is further purified by repeated solution in water and precipitation with alcohol. The amorphous, dry, white powder thus obtained contains only a part of the poison. No process is known by which it can be obtained in purity. The powder gives the characteristic proteid reactions. The poison is soluble in water, easily precipitated by alcohol and ammonium sulphate, is dragged down with fine precipitates, such as calcium phosphate, is only slightly dialyzable, is destroyed in solution at 58° C., when dry will stand 70° C., and in solution is destroyed by direct sunlight and weakened by exposure to diffuse daylight and oxygen. When inoculated into guinea pigs and rabbits, it produces all of the characteristic symptoms, including paralysis, and the lesions of diphtheria, with the exception of the false membrane. It is of appalling potency, according to Roux and Yersin four-tenths of a milligram sufficing to kill eight guinea pigs. When injected in a sufficiently small but fatal dose, the poison produces no apparent disturbance for days, and the death of the animal may occur weeks or months afterward with characteristic symptoms and lesions. This remarkable behavior, so unlike that which we are accustomed to attribute to chemical poisons, raises the question whether the poison may not be reproduced in the body after injection, or even whether the substance injected is itself the poison, and may not be something in the nature of an enzyme which leads to the production of the real poison within the body. These questions, which may be raised also regarding the tetanus poison, cannot be answered with our present knowledge.

It has been shown by Welch and Flexner that the diphtheria poison produces circumscribed areas of necrosis in various internal organs, and that the poison which causes the pseudomembrane is contained within dead diphtheria bacilli.

There are no bacterial infectious diseases other than diphtheria and tetanus, which have been reproduced in all essential features by inoculating animals with the chemical bacterial products, unless one chooses to consider as such the production of abscesses by dead bacteria or their products. We possess, however, knowledge of some of the bacterial products which are concerned in producing characteristic phenomena of other infectious diseases.

The filtrate of old liquid cultures of tubercle bacilli contains various proteids which are but slightly toxic for healthy persons, but are highly so for tuberculous human beings and animals, producing remarkable reactions and modifications of tuberculous lesions. They belong to the bacterioproteins which withstand boiling, and are derived from the bodies of the tubercle bacilli. Koch's tuberculin contains these various proteids. Masses of dead tubercle bacilli inoculated subcutaneously in animals cause local abscesses. Prudden and Hodenpyl have shown that suspensions of tubercle bacilli, killed by heat and thoroughly washed, produce when injected into the circulation of rabbits regular giant-celled tubercles, differing from tubercles caused by living bacilli in the absence of caseation, and of course of multiplication of the bacilli. The proteins of tubercle bacilli possess in high degree the property of attracting leucocytes. Prudden suggests that, in addition to these positively chemotactic tuberculoproteins, living tubercle bacilli produce in the body two other classes of poisons—one which causes caseation of tubercles, and another which causes the constitutional symptoms of tuberculous infection.

Mallein is a toxic protein of the glanders bacillus, bearing much the same relation to glanders that tuberculin does to tuberculosis.

Small quantities of virulent cultures of the cholera spirillum inoculated into the peritoneal cavity of guinea pigs cause death in a few hours, with symptoms of profound intoxication resembling those of the algid stage of cholera. According to the dose and virulence of the cultures and the susceptibility of the animal, the process may be a pure intoxication with only temporary and slight multiplication of the spirilla, or it may be an infection with marked reproduction of the spirilla.

The germ-free filtrate of fresh cholera cultures in bouillon contains little or no poison; that of cultures a month old contains a considerable quantity, probably extracted from the bodies of dead bacilli. The best known cholera toxins are those derived from the spirillum. These are highly poisonous, as is shown by the fact that the same profound symptoms of intoxication which follow the introduction of the living spirilla may be produced by the dead bacilli injected in slightly larger dose. These amorphous toxins incorporated with the bodies of the cholera spirillum are less stable substances than Buchner's bacterioproteins. Cultures subjected to high temperatures, however, are still poisonous. By cultivating the cholera spirillum in a medium free from proteid material, Brieger and Cohn have succeeded in obtaining an amorphous cholera poison devoid of the essential reactions of albuminous substances, with the exception of a slight response to Millon's reagent, which they attribute to admixture with a part of the substance of the bacterial cells.

Ordinary living cultures of the typhoid bacillus in tolerably large doses kill animals chiefly by intoxication—sometimes, however, when sufficiently virulent, by infection. According to Vincent, Sanarelli, and Chantemesse and Widal, the typhoid bacillus by combination with various bacteria, some saprophytic, or their products, may become so exalted in virulence as to produce in animals genuine infection, with extensive multiplication of the bacilli. Sterilized cultures of the typhoid bacillus are poisonous. Toxic proteids, which still retain considerable poisonous power after subjection to 100° C., can be extracted from the typhoid bacilli. A highly poisonous toxalbumin has been obtained by Brieger and Wassermann from the organs in two cases of typhoid fever in man. One-tenth of a gramme killed guinea pigs in twenty-four to forty-eight hours, with reduction of temperature, somnolence, and general prostration.

G. and F. Klemperer have given the name "pneumotoxin" to the poison produced by the pneumococcus (*Micrococcus lanceolatus*). We have no definite information as to the nature of this substance. Most investigators have had little success in determining the existence of definite toxic substances in cultures of the pneumococcus. As a rule, very large quantities of sterilized cultures or of the germ-filtrate of virulent cultures of this organism must be injected to obtain any evidence of toxicity. The pneumococcus is extremely variable in virulence, and some cultures are more poisonous than others. There is evidence, however, of the formation of substances of considerable toxicity in animals and in human beings infected with the pneumococcus. The blood in many cases of croupous pneumonia is in a high degree poisonous when injected into rabbits, and this toxic property does not disappear immediately after the crisis.

Various interesting toxic and immunizing proteids are produced by *Bacillus pyocyaneus,* an organism which sometimes causes general infection as well as local inflammation in human beings.

Cultures of the anthrax bacillus in bouillon are devoid of appreciable toxicity; those in defibrinated blood are somewhat toxic. Martin finds that in culture media containing a proteid, such as alkali albumin, capable of being digested by the bacillus, as well as in the living body, the anthrax bacillus produces poisonous albumoses. Hankin also finds albumoses in anthrax cultures of certain composition and at certain temperatures. Hankin's albumoses possess some immunizing and only slight toxic power.

Soluble toxic products are not abundant in ordinary cultures of the pyogenic cocci. Proteids capable of causing suppuration have been separated from these cultures. Rodet and Courmont have obtained substances, probably proteids, of moderate toxicity from old cultures of *Staphylococcus aureus.* We have clinical evidence that in some human infections with pyogenic staphylococci, and especially streptococci, toxic substances are formed. In accordance with this, Brieger and Wassermann obtained a toxalbumin from the bloody urine of a patient with facial erysipelas, and Stern found the blood of erysipelas patients toxic. Nissen found markedly toxic the germ-free blood of several patients presenting general symptoms from acute suppurative processes caused by the ordinary pyogenic staphylococci and streptococci. There are, indeed, many recorded instances of decided toxicity of the human blood, secretions, and excretions in various infectious diseases.

Capacity to produce local, especially suppurative, inflammation is a property common to a great many bacteria, and especially to such as are weakened in virulence or are introduced into relatively insusceptible individuals. This capacity depends upon the production by the bacteria of positive chemotactic substances; that is, of substances which attract leucocytes toward them. These chemotactic substances are believed to be, at least in many cases, of a proteid nature. Buchner believes that it is especially the bacterioproteins discharged from degenerating and dead bacterial cells which attract leucocytes, and that the specific toxic products are negatively chemotactic—that is, repel leucocytes. There is evidence, however, that living and vigorous bacteria, as well as dead bacteria, may attract leucocytes. Nor are all dead bacteria positively chemotactic. Massart believes that living bacteria secrete in varying degree special chemotactic substances, positive or negative according to their concentration, or perhaps distinct positive and negative substances, and that these substances are not identical with the toxic products. As a rule, virulent bacteria attract few leucocytes in a susceptible animal, but attract them in large number in an animal naturally insusceptible or rendered so by vaccination. To this rule there are exceptions.

Bacteria or bacterial products when injected into the circulation cause a speedy diminution in the number of leucocytes. In rapidly fatal infections this diminution is often extreme, and may continue until death. If recovery takes place, the diminution is generally followed sooner or later by increase in leucocytes beyond the normal number. In fatal infections of protracted course leucocytosis may or may not occur, there being often notable oscillation in the number of circulating leucocytes. When the bacteria produce local inflammations, there is generally a decided increase in the number of leucocytes in the blood. These experimental facts are in accordance with clinical observations in human beings, and indicate the prognostic value to be attached to leucocytosis, and especially to its absence, in certain infectious diseases, as has been demonstrated in pneumonia.

The phenomena of chemotaxis show that it is not only the distinctively toxic bacterial products which are capable of influencing infection. Under the head of Predisposition mention has been made of what may be called predisposing products of bacteria. These are products which, with or without causing manifest symptoms of intoxication, may render the individual receiving them more susceptible to infection, either to the bacteria producing them or to other bacteria. Ordinary saprophytes may produce such products. On the other hand, the products of some bacteria are antagonistic to infection with other bacteria.

The known facts concerning the biological properties, the causal relations, and the chemical products of bacteria have shed much light upon many pathological questions concerning infectious diseases. There remain, however, a vast number of unsolved problems. We must forego a further consideration of the general pathology of infections, as this would exceed the limits assigned to this article.

Immunity: Prophylactic and Curative Inoculations

Under Predisposition we have considered various conditions which influence susceptibility to infectious diseases. It remains now to consider in a more special way the subject of immunity.

Immunity from an infectious disease may be natural—that is, hereditary—or acquired. There are abundant instances of the natural exemption of certain animal species from diseases to which other, sometimes closely allied, species are susceptible. Field mice, for example, are resistant to mouse septicaemia, which is in the highest degree fatal to house mice, and, on the other hand, field mice are susceptible to glanders, to which house mice are refractory.

Prolonged, often lifelong, immunity is acquired by a single attack of certain infectious diseases, as the eruptive fevers, whooping cough, mumps, typhoid fever, yellow fever, and syphilis. There are other infectious diseases—for instance, diphtheria, pneumonia, and Asiatic cholera—an attack of which confers only temporary immunity. Some diseases, particularly erysipelas, leave behind after a time no increased insusceptibility, and there may follow even diminished resistance to subsequent attacks. There is evidence that some degree of insusceptibility, although it may be temporary and partial, follows an attack of most infectious diseases. The process of recovery from an infectious disease is closely related to the production of immunity.

Until Pasteur's discovery in 1880 of vaccination against chicken cholera, vaccination against smallpox was the only instance known of immunity acquired by this procedure. During the last dozen years means have been found of rendering susceptible animals more or less insusceptible to a large number of bacterial diseases. Thus immunity of varying degree has been experimentally produced in animals from the bacteria of chicken cholera, anthrax, erysipelas, symptomatic anthrax, malignant oedema, hog cholera, typhoid fever, haemorrhagic septicaemia, vibrionic septicaemia, Asiatic cholera, diphtheria, tetanus, pneumococcus infection, pyocyaneus disease, proteus infection, *Bacillus coli communis,* and the pyogenic staphylococci and streptococci. To this respectable list should be added rabies, the specific cause of which is unknown.

It is by these important discoveries that the problems of immunity have been partly removed from the domain of pure speculation and have been opened to experimental solution. Independently of their bearing upon the theories of immunity, the modern experimental studies of this subject have disclosed many new facts and points of view so interesting and important that they merit consideration in this work.

The principal means which have been employed to produce artificial immunity are as follows:

1. Inoculation of small, not fatal, quantities of living, virulent cultures of the specific microorganism causing the disease.

2. Inoculation of the living specific microorganisms partly or wholly attenuated in virulence. The ways by which virulence may be weakened or annulled have already been described (page 20).

3. Injection of products of the microorganism causing the disease. Use may be made of products contained in or obtained from sterilized cultures

or germ-free filtrates of cultures or of products obtained from the body of an infected animal. These substances may be injected when still toxic, or more frequently after diminution or removal of their toxicity. The principal means of reducing toxic power and increasing vaccinating power are heat and mixture with certain animal juices, particularly thymus extract, or with trichloride of iodine and some other agents injurious to bacterial poisons. The so-called chemical vaccines belong to this class. They have a very wide field of application. In some instances partial immunity has followed the introduction of this class of substances into the stomach.

4. Injection of the blood serum or other fluids from animals artificially rendered immune from the disease; also, also the ingestion of the milk of such animals. This method is different in principle from the preceding, and the resulting immunity presents important characteristics which will be explained later. As the blood serum from immunized animals may possess not only prophylactic but also curative properties, it has received the name of curative or healing serum.

The production of immunity by inoculation of a bacterial species other than that which causes the disease, or by the injection of chemical substances not derived from the specific bacteria of the disease, is of such limited application that it will not be considered here.

Frequently two or more of the principles of immunization described are combined in the method adopted. As a rule, various methods can be successfully employed in producing artificial immunity from a given disease.

Our understanding of the process of immunization was greatly advanced by the discovery that immunity, no less than disease, is produced by the chemical products of bacteria, and may be brought about by these substances, not only when they are elaborated after the introduction of the living micro-organisms in the body, but also when they are formed in cultures or in other animals. Hence the distinction between living vaccines and chemical vaccines is not so fundamental as at first glance might appear.

The bacterial products which produce immunity are generally believed to be proteids and to belong especially to that class of proteids which are derived from bacterial cells.

There is evidence that toxic proteids can confer immunity, but the fact that immunity can be more readily produced by sterilized cultures or filtrates, deprived by heat or in other ways of poisonous properties, than by the unaltered toxic substances has led to the view that the immunizing bacterial products are either distinct from toxic products or are derived from them. In large doses the vaccinating substances are generally more or less poisonous.

Immunity does not follow at once upon the introduction of the vaccinating substances into the body. On the other hand, the immediate effect of their introduction is sometimes increased susceptibility. There is a period of more or less pronounced reaction before immunity is established, and often the degree of immunity is proportionate to the intensity of the reaction. This period lasts usually several days, but it may be completed in a few hours or may extend to two or three weeks, varying according to the microorganism and the character and dose of the vaccine employed. If living vaccine be used, there are generally local inflammation and fever, and there may be a mild attack of the disease. It is not necessary that the bacteria introduced should multiply beyond the neighborhood of the point of inoculation. Fever and loss of weight are the most common symptoms of the reaction following the injection of chemical vaccines, which may or may not cause inflammation at the site of injection.

The immunity which is conferred from a disease by injecting into a susceptible animal the blood serum or other fluids of another animal which has already been rendered artificially immune from the same disease is contrasted in important particulars with that produced by vaccinating with the specific microorganism or its products. By the former method we introduce fluids which are already endowed with properties upon which immunity depends, whereas by the latter method these properties must first be developed before immunity is established. In accordance with this difference, we find that the blood serum from an artificially immunized animal protects at once or within a few hours after its introduction, and does not give rise to any notable reaction. This immediately protective effect of immune serum and the absence of disturbing reaction are qualities which suggested the use of such serum in the treatment of infections. The degree of protection afforded is in direct ratio to the quantity of immune serum introduced and to the degree of immunity possessed by the animal from which the serum was obtained. According to Ehrlich's conception, we simply transfer a part of the immunity possessed by one individual to another individual, and this second individual remains protected only as long as the immunity substances which have been introduced are retained within the body. Hence immunity conferred in this way is called by Ehrlich passive immunity, in distinction from the more stable active immunity produced by vaccination with microorganisms or their products. It is questionable, however, whether we are justified in taking quite so simple a view of the matter. The demonstration is not conclusive that in producing this so-called passive immunity immune serum or other immune fluids do not bring about some change in the body upon which the final immunity really depends. This change, however, whatever it may be, is certainly not comparable to that which leads to the development of active immunity with its antecedent reaction, often severe and prolonged over several days.

Passive immunity (to retain the use of this convenient but not wholly correct term) is of shorter duration than well established active immunity. It may, however, continue, with diminishing value, for at least two months. Active experimental immunity is of variable duration. When well established it may in the case of some diseases continue at least several years.

So far as the present evidence extends, it does not appear that any very substantial immunity can be produced by injecting the blood or fluids of naturally immune animals.

It is a general law that a given microorganism and its products are capable of conferring immunity only from that disease which is caused by this organism. An animal may, however, be vaccinated against two or more pathogenic microorganisms. The products of the tetanus bacillus, for example, vaccinate only against tetanus, so that an animal made immune from tetanus is not thereby in the least protected from diphtheria or any other infection than tetanus. The tetanus antitoxin is the product of a specific reaction of the animal body induced by products of the tetanus bacillus, and, so far as is known, can be produced only by this bacillus; and the same general statement, with some exceptions, is true of the other specific immunity substances. When, therefore, we find that two bacteria presenting many characters in common—for example, *Bacillus typhosus* and *Bacillus coli communis*—the products of the one will vaccinate it, but not against the other, the presumption is that the organisms are not identical. Moreover, when we find in animals or in human beings, after recovery from an infectious disease, specific immunizing substances which were not present before the disease, and which, so far as we know, can be produced only through the activity of a given microorganism, we feel justified in inferring that that microorganism has been operative in the causation of the disease. It is manifest that we possess in the detection of such specific immunizing substances a valuable method of demonstrating the casual relation of a microorganism to a given disease. This method has given positive results in human beings for diphtheria, tetanus, and croupous pneumonia. It has been found also that the blood of persons who have recovered from cholera or typhoid fever possesses decided, often very powerful, properties in protecting animals from the pathogenic effects of the cholera spirillum or the typhoid bacillus respectively. Thus Lazarus found in two persons who had recovered from cholera that one-tenth of a decimilligram of their blood serum, and Wassermann in one such case that $\frac{1}{100}$th of a decimilligram of the serum protected guinea pigs from the effects of subsequent inoculation with a quantity of cholera bacilli surely fatal to unprotected guinea pigs. The protective influence of the blood progressively increased in Wassermann's case for seven weeks after recovery from cholera. Similar protective properties of human blood against the typhoid bacillus have been found after recovery from typhoid fever. These observations are very significant, but their force is somewhat lessened by the fact that the blood of persons who have never had cholera or typhoid fever sometimes possesses similar protective properties, although less constantly and generally in less degree than in the case of those who have recovered a short time previously from these diseases.

A principle of great importance is that immunity may be augmented in degree by the gradual employment of vaccines of increasing strength, followed by the introduction of the virulent microorganisms or their products, until finally sometimes doses vastly larger than suffice to kill unvaccinated animals can be inoculated. These very high degrees of insusceptibility, however, have been attained only in the case of those toxic diseases, such as diphtheria and tetanus, the immunity from which depends upon an antidotal power of the blood and fluids against the specific poison of the disease.

When we come to test experimental immunity from various microorganisms by injecting into the protected animal these organisms or their poisonous products, we find that there are two principal kinds of insuspectibilty. With one kind the animal is protected against a fatal dose of the specific bacteria without possessing any decidedly increased tolerance of the toxic products of the bacteria. According to some observations, immunity of this kind is in some instances associated even with diminished tolerance of the poisons. This immunity must depend upon restraint of growth or destruction of the bacteria or upon deprivation of their power of producing toxic substances.

With the other kind of immunity the protected animal is insusceptible to the poisonous products of the specific organism, so that even if the organism should survive for a time in the body, it is reduced to the level of an ordinary saprophyte. It has been proposed to limit the term "immunity" to the first kind, and to describe as "poison proof," but not immune, those individuals who are simply insusceptible to the poison. While recognizing the importance of the distinctions mentioned, it does not seem wise at present thus to limit the signification of the word immunity. Our knowledge of the conditions underlying immunity is not sufficient to determine how fundamental these distinctions really are.

A moderate degree of insusceptibility to the specific poisons of bacteria may be acquired as the result of a simple tolerance of these poisons, but when this insusceptibility exists in a high degree after immunization it is due to the power of the blood and other fluids of the immune body to antagonize these poisons. If the specific poison be mixed outside of the body in proper proportion with the immune blood serum, it is found that this mixture can be injected into a susceptible animal without manifesting poisonous properties. It has been generally inferred from this observation that the fluids of the immunized animal actually destroy or neutralize the poison. Such fluids are therefore said to be toxicidal, in distinction from bactericidal, and the antidote to the poison is called antitoxin. It has not, however, been proven that the antitoxin acts by destroying the toxin. It may be that both act in an antagonistic manner upon the living cells of the body, the antitoxin rendering the cells insusceptible to the toxin. Experiments of Buchner indicate the posibility of this latter explanation.

We have no definite knowledge of the chemical nature or the source of these antitoxins. They are commonly believed to be proteids of some form. They are less susceptible to the action of heat and other injurious agents than are the so-called toxalbumins. The albuminous precipitates thrown down from antitoxic fluids by alcohol, and more especially those obtained by adding ammonium sulphate or magnesium sulphate, retain in the dried state a considerable part of the antitoxin. In various ways antitoxins may be obtained in a more concentrated form than in the original fluids.

Our principal knowledge of the practically and scientifically important subject of antitoxic immunity relates to tetanus and diphtheria, and to the vegetable toxalbumins, arbin, ricin, and robin, studied by Ehrlich. We owe to Behring and Kitasato and to Ehrlich the pioneer investigations in this line. The primary immunization of susceptible animals from tetanus or diphtheria may be accomplished in various ways by the bacilli or their products partly deprived of virulence by heat, trichloride of iodine, or other agents.

A most important characteristic of artificial immunity from toxins is that by successive injections of increasing amounts of the poisonous substances into the vaccinated animals the antitoxic or immunizing power of the fluids can be augmented to an astounding degree. It is in this way that the immunizing power of the fluids may become sufficiently high to exert curative effects when injected after the introduction into a susceptible animal of the specific microorganism or its poison. Each fresh injection of the specific poison into the animal undergoing immunization uses up a certain amount of antitoxin, but this primary reduction is followed each time by a rise in antitoxic power. A partial decline follows this elevation, but the curve remains at a level higher than that existing before the injection. This reaction before the permanent level is reached occupies several days. Considerable time, therefore, is required to obtain blood of very high immunizing power. To what height this may reach we cannot at present tell. Behring in 1892 estimated, according to his method, the immunizing value of the blood serum of a horse which he had during two years rendered increasingly immune from tetanus as 1 to 10,000,000, by which is meant that $\frac{1}{10000000}$th c. c. of serum will protect 1 gram-weight of mouse from the subsequent injection of the smallest, surely fatal, dose of the tetanus poison, or 1 c. c. of serum suffices to immunize 500,000 mice, weighing each 20 grams, or 200 sheep, weighing each 50 kilo.

Artificial immunity from rabies probably depends upon this so-called antitoxic power of the blood and fluids of protected animals, and, as is the case with acquired immunity from tetanus and diphtheria, this power may be increased by successive inoculations with the virus of rabies.

To what extent acquired immunity from other infectious diseases affecting human beings depends upon an antitoxic power of the blood cannot at present be stated. It has been claimed by the Klemperers that this is the explanation of immunity from *Micrococcus lanceolatus,* which causes croupous pneumonia. Their observations on this point are not in accord with those of several other experimenters. The experimental immunity which can readily be produced in animals from the bacteria of cholera and of typhoid fever has also been referred to the development of an antitoxic power of the blood, but recent investigations indicate that animals protected by vaccination against these bacteria are not insusceptible in any marked degree to their poisons.

The blood of animals protected by vaccination from the pneumococcus, the cholera spirillum, or the typhoid bacillus is capable of protecting susceptible animals from subsequent inoculation with these microorganisms. This protective power of the blood reaches the highest degree in cases of antitoxic immunity, which is unattended by insusceptibility to the specific poisons of the casual microorganism. It seems to belong to any solid acquired immunity.

There are many infectious diseases acquired immunity from which cannot be explained by the existence of an antitoxic power of the blood analogous to that present in artificial immunity from diphtheria and tetanus. Immunity from anthrax and other septicaemic diseases, accompanied by great invasion of the blood with bacteria, cannot be explained in this way. The antitoxic principle does not furnish an explanation even for natural, hereditary immunity from tetanus and diphtheria. The blood of the hen, which is naturally immune from tetanus, is not an antidote to the tetanus poison, but it may acquire this antitoxic power after injection of this poison, notwithstanding the fact that the hen is not susceptible to it. This is an illustration of the fact that tolerance by the body cells of specific bacterial poisons may exist without any antidotal power of the blood and fluids against these poisons. It also shows the wide difference there may be between the basis of natural immunity and that of acquired immunity from one and the same disease. The same general statements are true as regards rats and diphtheria.

Difficulty has been found in conferring immunity from diseases caused by the multiplication of bacteria in the intestinal canal. Guinea pigs and rabbits vaccinated against cholera spirillum are protected against several times the fatal dose of cholera cultures introduced into the peritoneal cavity, but it is not satisfactorily established that they are protected from infection with cultures introduced by way of the stomach and intestine under the conditions in which this mode of introduction causes infection. Infections and intoxications of animals with cholera spirilla or typhoid bacilli inoculated into the peritoneal cavity are so different from the natural diseases in human beings that we must be very guarded in applying to the latter these experimental results.

The discovery by Ehrlich that immunity may be afforded by the ingestion as well as the injection of the milk of immunized animals is an important contribution to our knowledge of immunity and to our resources in the way of prophylaxis and treatment. It bears upon the question of inheritance of acquired immunity.

Acquired immunity may be transmitted from the mother to her offspring. The question of germinal transmission of acquired immunity not only is of medical interest, but bears also upon the great problem of inheritance of acquired traits. Only paternal inheritance can give a conclusive answer to this question, at least so far as mammals are concerned. In the case of inheritance of acquired immunity from the mother, the possibility of transmission through the placenta rather than through the ovum cannot be excluded.

The only instance known to the writer in human beings in which it has been claimed that artificial immunity may be acquired by germinal inheritance is that embodied in Profeta's law, which asserts that healthy children born of syphilitic parents are protected against syphilis. The evidence, however, is certainly inconclusive that the healthy child of a syphilitic father and a nonsyphilitic mother enjoys any increased insusceptibility to syphilis. The experimental data bearing upon this question are too meagre to furnish a definite answer. Ehrlich found that mice born of normal mothers impregnated by males rendered highly immune from the toxic phytalbumoses, abrin and ricin, possessed no immunity whatever from these substances, and he therefore concludes that "the idioplasm of the sperm is not capable of transmitting immunity." On the other hand, Tizzoni and Centanni assert that acquired immunity from rabies may be transmitted, although much lessened in degree, from the immunized father to the offspring of mothers unprotected against this disease, and Tizzoni and Cattani claim to possess the same evidence for tetanus. They interpret these results as in support of the doctrine of inheritance of acquired traits. Similar experiments by other investigators have afforded negative results.

Passing to placental inheritance, we have to consider three possibilities: (1) protection afforded by contracting the disease in utero; (2) protection resulting from the reception of vaccinating substances from the mother (active immunity); (3) protection conferred by the reception of developed immunity substances from the mother (passive immunity). There is no doubt as to the occurrence of the first and third modes of protection. We have already considered sufficiently intra-uterine foetal infection, which does not bear upon the question of inheritance of acquired immunity. The occurrence of immunity as the result of reception by the foetus of immunity substances contained in the mother has been demonstrated by Ehrlich for immunity from abrin, ricin, and robin, and by Tizzoni and others for tetanus and rabies. This passive immunity is reinforced after birth by ingestion of the mother's milk, which possesses the same protective properties as the blood. By this reinforcement it continues longer than would otherwise be the case. The observations concerning inherited artificial immunity in human beings from smallpox, syphilis, and, doubtfully, vaccinia, and in animals from sheep-pox, anthrax, symptomatic anthrax, pneumococcus infection, infection with the typhoid bacillus, and some other diseases, have not generally been made under conditions which permit a positive decision as to whether the immunity is the result of vaccination of the foetus coincidentally with that of the mother, or of the simple transmission of developed immunity from mother to foetus, subsequently reinforced by the mother's milk. When the mother's immunity is induced during pregnancy by recovery from infection or by vaccination with bacterial products, it does not necessarily follow that acquired foetal immunity is due also to vaccination. It may be so-called passive immunity. The duration of the inherited immunity may afford an answer to this question.

The only instance known in which immunity appears to be received by the mother from the foetus is that sometimes observed in the healthy mothers of syphilitic infants (Colles' law).

We possess no general explanation of the basis of immunity. It is certain that the various kinds of immunity—the immunity of plants and of animals, the immunity of warm-blooded and of cold-blooded animals, the immunity from ordinary obligate saprophytes and that from pathogenic organisms, immunity from bacterial poisons and that from the bacterial cells alone, natural immunity and acquired immunity—do not all depend upon the same causes. We can give space here to a brief consideration only of those theories of immunity which rest upon some experimental basis of facts.

The leading theories of immunity which more especially concern us may be brought into two classes—one which attributes immunity to the direct and active intervention of the living cells of the body, and the other, which explains immunity by the properties of the extracellular fluids of the body. In the last analysis these properties of the body humors must depend upon the activities of cells, so that we must have recourse either directly or indirectly to cellular functions in any adequate explanation of immunity.

The chief representative of the first class of theories is the phagocytic theory, which has been elaborated by Metchnikoff with a wealth of detail and a clearness and charm of presentation which make this theory the most fascinating and satisfying to the mind of all. It has also the advantage of a wider applicability, if accepted, than any other explanation of immunity.

The phagocytic theory supposes that immunity depends upon the seizure of invading microorganisms by amoeboid cells of the body, and the subsequent destruction of these organisms by digestion or other chemical agencies in the interior of the cells. The polynuclear leucocytes are the most numerous and important phagocytes, but other mesodermic cells may perform the same function. According to this view, the phagocytes are charged with the defence of the body, and engage in a veritable conflict with the parasites. Immunity is acquired when the phagocytes have gained tolerance of the poisons of microorganisms and are no longer repelled by them.

The main support of the phagocytic theory is found in the difference in behavior toward invading microorganisms between the leucocytes of a susceptible and those of an insusceptible individual. If the individual is insusceptible, phagocytes, chiefly leucocytes, accumulate around the microorganisms and take them up, whereas in a susceptible individual this phagocytosis is generally absent or present only in small degree.

Undoubtedly there exists as a general law, to which there are not a few exceptions, this parallelism between phagocytosis and immunity. But in order to prove that insusceptibility is to be explained solely or chiefly by phagocytosis, it must be shown that the microorganisms have not first been injured by extracellular agencies before their reception by phagocytes, and also that, even if living and virulent microorganisms are seized and destroyed within cells, they may not also be weakened or killed outside of the cells.

In the opinion of the writer these objections have not been overthrown by the supporters of the phagocytic hypothesis. It is true that Metchnikoff has proven that phagocytes may take up living and apparently virulent bacteria, and that these bacteria undergo degeneration and death in the interior of the cells. But he has not shown that, as a rule, bacteria when taken up by cells have suffered no injury from extracellular agencies; and, inasmuch as we know that fluids of the body may damage bacteria, the phagocytic theory must bring evidence of the exclusion of these agencies. Direct observations which show that bacteria which have been introduced into the body may degenerate and die not only within cells, but also outside of them in the humors and tissues, are many and come from good investigators, and, although vigorously contested by Metchnikoff, must be accepted.

The principles of chemotaxis have been urged both for and against the phagocytic theory. Those who hold with Buchner that it is chiefly or exclusively the proteids derived from degenerating and dead bacteria which attract leucocytes, whereas the toxic proteids repel them, consider that this is the strongest argument against the phagocytic theory. But, as has already been mentioned, there is evidence that also living and vigorous bacteria may secrete positively chemotactic substances, so that we are not inclined to give decisive weight to this line of argument.

It has been urged against the phagocytic theory that in chronic infections like tuberculosis and leprosy, and in some acute infections, notably mouse septicaemia, living bacteria multiply and flourish in large numbers inside of leucocytes and other cells, and often destroy these cells. The supporters of the theory reply that it is not enough that parasites should be simply received by leucocytes: the intracellular agents of destruction must be operative. The phagocytes have done their best, but these bacteria adapt themselves to the medium within the cells and triumph over them.

We cannot therefore assign to phagocytosis the exclusive rôle which is given to it by Metchnikoff in the explanation of immunity. That it plays a part cannot be doubted; the only question is, How important a part? To regard the phagocytes simply as scavengers and crematories for dead microorganisms is not in accordance with the undoubted fact that they may seize and destroy living bacteria. Some think that the phagocytes complete the work of destruction already begun outside of them; others believe that microorganisms perish equally inside and outside of the cells, the conditions being perhaps more unfavorable for them within the cells; and others hold that the antibacterial properties of the extracellular fluids are produced or increased through the agency of leucocytes and other cells. Kossell believes that nucleinic acid contained in or derived from leucocytes and other cells is the chief bactericidal agent. It has been held that leucocytes may form a wall around microorganisms and mechanically hem them in.

There are many theories which attempt to explain immunity by the properties of the body humors.

It has been urged that immunity may depend upon a lack of nutritive material capable of assimilation by the microorganism in question, which therefore dies of innutrition. This explanation may apply to some instances of natural immunity—for example, from ordinary saprophytes—but experiments have made it impossible to accept it as the explanation of most cases of immunity, especially of acquired immunity.

The process of immunization and the phenomena of immunity already described necessitate the rejection of the retention hypothesis, at least in its original form, according to which acquired immunity was thought to depend upon the retention for an indefinite period within the body of products of the microorganisms from which protection has been acquired.

The power of the fluids, particularly the blood, of destroying or antagonizing the action of the specific poisons of microorganisms is the explanation of acquired immunity from a certain number of toxic diseases already considered. Indeed, this antitoxic power is the most thoroughly demonstrated explanation of immunity which we have, but it is applicable only to a limited number of diseases—exactly how large a number we cannot at present say—and apparently only to acquired immunity.

Of the humoral theories of immunity, the one which deserves most consideration for diseases in which the antitoxic power of the blood does not come into question is that which attributes immunity to a direct germicidal action of the blood and fluids of the body. Since the important investigations of Nuttall upon the power of the blood and other fluids to kill bacteria in a short time, this theory of immunity has had many supporters.

Buchner has given the name of " alexins," and Hankin that of " defensive proteids " to the bactericidal and antitoxic substances of the blood and body fluids. Bactericidal alexins are believed to be proteids of an unstable nature. They lose their characteristic property when deprived by dialysis of their combination with certain salts. Vaughan has shown that solutions of nuclein are powerfully germicidal. According to Kossell and to Vaughan, the most important bactericidal agent in the body is to be found in nucleinic acid or in nucleins. The bactericidal power of blood belongs to the serum independently of the presence of cells, and is tested by mixing with the sterile blood serum or other fluids outside of the body a known number of bacteria, and determining quantitatively, by well established methods at definite intervals of time, whether the bacteria have multiplied or been arrested in development or have died. It is found that the blood serum varies in bactericidal power according to the animal from which it is derived, and according to the species of bacteria with which it is tested. According to these varying conditions there are all grades of action, from entire absence of bactericidal power to a capacity of killing many hundred thousand bacteria within a few hours.

The chief difficulty with this theory of immunity has been to establish any such correspondence between insusceptibility and bactericidal power of the blood and fluids as exists between phagocytosis and immunity. We find, on the one hand, that the blood serum of some animals susceptible to a given bacterium is destructive in marked degree to this organism, and, on the other hand, that the serum of some animals insusceptible to a bacterial species is devoid of notable bactericidal power over this species. There are many cases in which we cannot at present explain immunity by any demonstrated antibacterial action of the blood and fluids, but this is not saying that such action is absent in these cases.

There are, however, several striking instances of correspondence between immunity and bactericidal power of the blood. This is true in some cases of natural immunity, but more convincing are those cases in which the bactericidal capacity is developed as an accompaniment of acquired immunity. The blood serum of normal guinea pigs possesses relatively little bactericidal power over *Vibrio metchnikovi,* but that of guinea pigs rendered by vaccination insusceptible to this organism has acquired the power of killing in a short time large numbers of the vibrio. A similar difference has been demonstrated, although less constantly, between the blood of normal guinea pigs and that of guinea pigs vaccinated against cholera vibrio, which is very similar in morphology and biological properties to the vibrio of Metchnikoff. Metchnikoff attempts to break the force of these observations by the contention that the bacteria may indeed be killed by the blood serum outside of the body, but that they are not killed by the blood and fluids in the living body. The conditions are of course different in the living body from those in the test tube, but there is evidence that the bactericidal power of the fluids may be exerted within the body, although often less completely and less rapidly than in the test tube.

It is permissible to suppose that antibacterial properties may pertain to the extracellular fluids within the living body, when they are not always demonstrable in fluids withdrawn from the body, and also that these properties may be latent and developed in the immune individual as the result of a reaction set up by the invasion of the bacteria; but we have little positive evidence to support these suppositions.

It has been claimed that immunity may sometimes depend upon other properties of the fluids besides the bactericidal and antitoxic, such as simple restraint of growth or attenuation of virulence or deprivation of the power of producing toxic substances.

We have considered, thus far, the protection which may be afforded by immunizing substances when introduced into the animal body before the entrance of pathogenic microorganisms. We have seen that as regards many important microorganisms of disease there is no especial difficulty in conferring upon animals greater or less insusceptibility to subsequent infection. When, however, the attempt is made to give protection by these substances after pathogenic organisms have been introduced into the body, the difficulties in attaining success become greater and greater as time elapses, and after the symptoms are well marked the difficulties are vastly greater in effecting cure than they are in simple immunization before infection.

Curative substances have been sought for among the cultural products of bacteria. Koch's tuberculin is a notable example. The specific vaccinating substances have been applied to the treatment of experimental infections. There are, however, serious obstacles to attaining success in healing with these bacterial products. These vaccinating substances require, as a rule, considerable time, two or three days, and usually longer, to produce immunity. They must therefore be obtained generally in a more concentrated form if they are to be used for acute infections. In some instances such concentration has been accomplished. This class of bacterial products, unless completely deprived of toxic properties, is likely to produce a reaction which is distinctly unfavorable, and may increase susceptibility to the microorganisms forming them. In a few instances this difficulty also has been overcome. The successes, however, which have been attained in animals with concentrated immunizing and nontoxic bacterial products injected after the entrance of the microorganisms into the body have been only modest, and consist chiefly in conferring protection when the treatment has begun before the manifestation of distinct symptoms or in infections of slow progress and only moderate intensity.

Greater success has been reached in treating experimental infections or intoxications by means of the blood serum, milk, or other fluids from animals which have been rendered by vaccination immune from the microorganisms causing the disease. This method of treatment is called blood serum therapy by Behring, who has been especially active in developing its principles. The characteristic properties of this curative serum have already been described.

The principles of blood serum therapy have been worked out most fully for tetanus and diphtheria. It has been found that the curative properties of the serum are in direct proportion to the quantity injected and to the degree of immunity possessed by the animal yielding the serum. To produce the same curative effects in large animals as in small it is necessary to inject proportionately larger amounts, and as regards tetanus these amounts stand in approximately direct ration to the respective weights of the animals. Of decisive influence upon the result are the stage and intensity of the disease. If the serum be injected immediately after the reception of the virus, a larger quantity of serum is required to afford protection than a few hours previously, and the amount required increases as time elapses, until as soon as the very first symptoms of the minimal fatal dose of the tetanus poison appear in the mouse, one thousand times the quantity of serum necessary for simple immunization must be injected, and with each succeeding hour the requisite amount increases at an enormous rate, so that after twenty-four hours the necessary quantity of serum possessing an immunizing value of 1 to 1,000,000 is too great to be introduced into the animal (Behring). The larger the amount of tetanus poison which has been received, the greater must be the quantity of healing serum injected.

A desideratum, as yet only imperfectly attained, is a method of obtaining the specific curative substances in a much more concentrated and easily preserved form than in the original fluids.

The conditions have been found, as regards progressive increase of dosage of serum according to the stage of the disease, decidedly more favorable for experimental diphtheria than for tetanus.

It is evident that the difficulties to be overcome in the successful application of serum-therapy to large animals and to man are great. It is important not to generalize, but to work out for each disease separately a sound experimental basis for this mode of treatment, as has already been done with considerable success for tetanus. This statement is sufficiently evident when we consider the varying conditions which underlie immunity from different diseases, the varying degrees of immunity attainable, and our imperfect knowledge as to both of these points as regards many diseases.

PRELIMINARY REPORT OF INVESTIGATIONS CONCERNING THE CAUSATION OF HOG CHOLERA[1]

Investigations of the epizootic diseases of swine, occurring in the neighborhood of Baltimore, have been made by the writer, with the cooperation of A. W. Clement, V. S., and F. L. Russell, V. S.,[2] in the Pathological Laboratory of the Johns Hopkins University during the past two years. We have examined about fifty hogs, from six herds, affected with hog cholera, as well as several isolated cases. In this communication, which must necessarily be brief, only a summary of the most important results will be given, a fuller report being in preparation for the volume of studies from the Pathological Laboratory, to be issued by the Johns Hopkins Hospital.

The most common and characteristic lesions consisted in superficial and deep necroses, either circumscribed or diffuse, of the inflamed mucous and other coats of the large intestine, associated often with superficial branny diphtheritic exudation. Similar necroses were occasionally found in the stomach and small intestine, in the mouth, palate and epiglottis, and less frequently in the gall bladder, bile ducts and preputial sac. Some form of pneumonia was usually, although not constantly, present. In a few cases pneumonia was present without intestinal lesions, more frequently intestinal lesions were observed with little or no pneumonia. Strongyles in the bronchi were rarely missed. Bronchitis was the rule. Pleurisy was common; pericarditis and peritonitis were present in the minority of cases. Redness of the skin was common, but inconstant. The subcutaneous, mediastinal and abdominal lymph glands were usually swollen and reddened, chiefly in the periphery. The spleen was often normal, but in many cases was moderately and sometimes extremely swollen. The kidneys were either normal or the seat of haemorrhages and of parenchymatous degeneration or nephritis. The liver was often normal, but sometimes it presented necrotic areas. Ecchymoses were often observed in the gastric and intestinal mucosa and beneath the epicardium and endocardium. In some cases all of the organs of the body were studded with small haemorrhages.

The bacteriological examination consisted in the study of cover-glass preparations from the different parts of the body, in the inoculation of animals, either white mice or rabbits, with parts of the lung, spleen, liver intestine and sometimes other organs, and in the preparation of Esmarch roll cultures, usually of agar, from the blood, intestinal contents and all of the principal organs of the body.

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[1] Johns Hopkins Hosp. Bull., Balt., 1889-90, I, 9-10.

[2] F. L. Russell was associated with us in the work for about one year.

Of the bacteria isolated in pure culture and observed in microscopical preparations of the tissues only two species were sufficiently common or had such distribution as to suggest an etiological relation to the disease. These are the so-called hog-cholera bacillus and the swine-plague bacillus, the former first described in the Report of the Bureau of Animal Industry for 1885 as the bacterium of swine plague and in the report for 1886 as the bacterium of hog cholera, a change of nomenclature due to the detection in certain diseased swine in this country of the latter organism which now received the name of the bacterium of swine plague, as it was believed to be identical with the microorganism previously described by Löffler and by Schütz as the specific cause of Schweine-seuche in Germany.

The bacilli of hog cholera are short rods with rounded ends, averaging 1μ—2μ in length and about 0.6μ in breadth, but forms both longer and shorter than these measurements may occur. They are very actively motile. They grow readily on all of the ordinary culture media and best at temperatures between 30° and 38° C. They do not liquefy gelatine. The growth on gelatine and on agar has a grayish or whitish color, often with a bluish translucence. Bouillon cultures present a diffuse cloudiness with whitish sediment and without surface membrane. The growth on potato assumes generally a brownish or yellowish tint, but it may be white, and sometimes is indistinct, although microscopically the growth is abundant. The bacilli are killed by exposure for 10 minutes to a temperature of 58° C. In cover-glass preparations, from the fresh juices and tissues of animals dead of hog cholera the bacilli stain readily, and for the most part uniformly, with aniline oil gentian-violet. If the stained specimen be treated with acetic acid, many of the bacilli appear with clear centre and stained margin, which may be either uniform or slightly thicker at the poles, as described in the Reports of the Bureau of Animal Industry. Some may present a typical polar staining, but we do not regard them as good polar staining bacilli, like those of swine plague. Various irregularities in staining appear in old cultures.

The hog-cholera bacilli are pathogenic for rabbits, mice, guinea pigs and pigeons. We shall describe here only the experiments with rabbits. These animals when inoculated subcutaneously with a platinum loop for a pure culture of hog-cholera bacilli die usually in from 6 to 8 days, but the duration of life may be shorter or longer. There is generally considerable dry purulent infiltration at the seat of inoculation, the subcutaneous lymphatic glands on the same side are enlarged and often present necrotic foci; the spleen is swollen, as a rule extremely, and of a dark red color and firm consistence, the liver generally presents yellowish white streaks and dots, the heart muscle is fatty and in some cases ecchymoses, necrotic patches and diphtheritic exudation may be found in the intestinal mucosa. The bacilli, which often occur in clumps, are found most abundantly at the seat of inoculation, in the affected lymph glands, the spleen and the liver, and are often so scanty in the blood as to escape detection by microscopical examination. We have confirmed the statements in the Reports of the Bureau of Animal Industry of the effects of these bacilli when inoculated in pigeons.

The swine-plague bacilli are shorter than the hog-cholera bacilli. Measuring on the average 0.8 to 1.4μ in length, they may be very small and present the appearance of slightly oval bodies more like cocci than bacilli, or on the other hand they may present themselves as rods of considerable length. In appearance and other properties they belong to the same group of organisms as the well known bacteria of chicken cholera and of rabbit septicaemia. They are devoid of independent motion. They grow on the ordinary culture media, with the exception of potato, but at ordinary temperatures the growth is less rapid and abundant than that of the hog-cholera bacilli. They do not liquefy gelatine. On gelatine and agar the growth is grayish, translucent, not extending far from the point of inoculation. Bouillon cultures are sometimes diffusely cloudy but more frequently the growth is in the form of a whitish, rather viscid sediment, or in little specks, with clear fluid. When planted on potato there may be a feeble invisible growth for one or two generations, probably due to the transference of a little nutritive medium to the potato with the organisms. We have not been able to cultivate them for several generations upon potato. They are killed at a temperature slightly lower than that destructive to hog-clohera bacilli and their vitality in cultures in much shorter than that of hog-cholera bacilli. In cover-glass preparations from the fresh juices and tissues of animals dead of swine-plague inoculations the bacilli present an exquisite and typical polar staining, unless the forms are very short, when the staining is uniform. They are pathogenic for rabbits, mice, guinea pigs, pigeons and bats. We have met two degrees of virulence in this organism. The one kind kills rabbits in from 16 to 30 hours with enormous multiplication of the bacilli in the blood and organs; the other kind destroys life in from 2 to 6 days, occasionally longer, with extensive purulent and serous infiltration at the seat of inoculation, often with peritonitis, and with frequently few bacteria in the blood and organs but an immense number in the inflammatory exudates.

Regarding the distribution in the diseased hog of these two species of bacteria, great variety exists, which cannot be fully described in this short communication. In some cases the hog-cholera bacilli have been found abundantly in the blood, intestine and all of the organs, in other cases they have been present only in certain parts, most frequently the spleen and liver, and absent in other parts. They may be absent from the spleen when abundant elsewhere, as in the kidney.

The swine-plague bacilli, when present, likewise vary in different cases in their distribution. They are most frequently found in hepatized areas in the lungs, but they may also exist in the intestine, the blood and various organs.

As regards the frequency with which each of these organisms has been found in the diseased hogs we have met the following groups of cases: first, herds of diseased swine in which only the hog-cholera bacillus has been found; second, herds in which only the swine-plague bacillus was present; third, herds in which both the hog-cholera bacillus and the swine-plague bacillus were present in the same animal, or the hog-cholera bacillus in some animals and the swine-plague bacillus in others of the same herd. We have met a few, chiefly scattered, cases in which neither the hog-cholera nor the swine-plague bacillus was found.

We have not been able to establish any constant anatomical differences between the cases in which the swine-plague bacilli alone were present and those in which only hog-cholera bacilli or both organisms were found. While we have frequently found only the swine-plague bacilli in extensive hepatized areas in the lungs, we have also sometimes found the hog-cholera bacilli alone in apparently similar pneumonias. We have not met any epizootic corresponding to the German Schweine-seuche in which pneumonia existed in any large number of cases without intestinal lesions.

With these results we naturally looked with especial interest to the effects of inoculation of healthy hogs with pure cultures of each of these organisms. The most stringent precautions were taken in the selection and care of the experimental hogs.

Two hogs, weighing about 75 pounds, not subjected to any preliminary treatment, were fed each 225 c. c. of bouillon culture of hog-cholera bacilli. The one died in 4 and the other in 8½ days with extensive diphtheritic inflammation and superficial circumscribed necroses of the large intestine, with moderate swelling of the spleen and of the lymphatic glands, and with ecchymoses in the lungs and elsewhere. Strongyles were present in the bronchi but there was no pneumonia. Hog-cholera bacilli were found in abundance in the blood, intestine and organs. In a third hog 6.5 c. c. of the same bouillon culture were injected with antiseptic precautions into the duodenum. Death occurred in 7 days with the same lesions as in the preceding hogs. Two hogs exposed in the same pen with the first hog were sick for a number of days and gradually recovered. These, when killed, presented undoubted evidence of the previous existence of acute diphtheritic inflammation of the large intestine.

The injection into the thigh and into the lung of 5 c. c. of the same bouillon culture in two other hogs produced only localized sloughs with slight constitutional disturbance. The hogs were killed at the end of five weeks and hog-cholera bacilli were found alive in the sloughs but none elsewhere in the body.

The injection into the right lung of a pig of 8 c. c. of a pure bouillon culture of swine-plague bacilli was followed in 48-60 hours by death with extensive pneumonia, double fibrinous pleurisy, pericarditis and peritonitis and with very abundant swin-plague bacilli in the exudates, the blood and the organs. Intestinal lesions were absent. The injection of 0.5 c. c. bouillon culture of swine-plague bacilli into each lung of another pig was followed by great rapidity and difficulty of respiration and coughing. The animal was killed at the end of a week. Double serofibrinous pleurisy and pericarditis and foci of pneumonia were found. The swine-plague bacilli were present in abundance. The injection of pure cultures of swine-plague bacilli with a fine hypodermic needle into the peritoneal cavity was not followed by any manifest effects, but in two cases in which laparotomy was performed with antiseptic precautions and pure cultures of swine-plague bacilli (6.5 c. c.) were injected into the duodenum the animals died in from 16 to 30 hours with acute diffuse peritonitis, pleurisy and pericarditis and enormous number of swine-plague bacteria in the exudates, blood and organs, but without intestinal lesions. Doubtless some of the culture escaped into the peritoneal cavity. Subcutaneous inoculations in two cases and feeding in four cases of swine-plague cultures produced no lesions, save localized abscesses and sloughs after the injections.

It is evident from these experiments, which, together with others, will be reported more fully hereafter, that both the hog-cholera bacilli and the swine-plague bacilli are pathogenic for swine, that the former when fed or injected into the duodenum, even in comparatively small quantity, are capable of producing intense diphtheritic inflammation and necrosis of the large intestine with general infection and the latter when injected into the thoracic cavity or into the injured peritoneal cavity of causing pneumonia and inflammation of serous membranes.

If, as seems probable, from these observations and experiments the hog-cholera bacilli are to be regarded as the cause of hog cholera, at least of the intestinal lesions, how are we to explain the failure to find these bacilli in a number of cases of the disease. A number of possibilities suggest themselves. First, the bacilli may be confined to the intestine and mixed with so many other bacteria that it is difficult or impossible to isolate them. Their morphology and the appearance of their colonies are so little characteristic that this might readily happen. That this, however, cannot always be the explanation, is evident from the fact that, in several instances, rabbits inoculated with typical necrotic buttons have survived, and cultures and inoculations from other organs have failed to reveal the bacilli of hog cholera. Second, the bacilli may be confined to the intestine, and so modified that they fail to kill rabbits when inoculated subcutaneously. These bacilli appear to vary somewhat in their virulence and the possibility suggested cannot at present be disproven. Third, as in cases of typhoid fever and croupous pneumonia in human beings, the specific bacilli may disappear in the later stages of the disease. This explanation, which is suggested in the Reports of the Bureau of Animal Industry, seems to us probable, but as already mentioned we have not been able to distinguish anatomically cases in which hog-cholera bacilli could not be detected from some of those in which they were present.

It is not clear to us what rôle is to be assigned to the swine-plague bacilli in the natural infections which we have studied. The facts that experimentally the swine-plague bacillus is capable of causing extensive pneumonia and inflammations of serous membranes and that epizootics occur in swine in Germany with these as the predominant lesions without intestinal disease, suggest that this organism, which is apparently identical with that of the German Schweine-seuche, is also the cause of a similar affection in this country. We are not, however, aware that any swine epizootic of pneumonia without any intestinal lesions and with the sole presence of the swine-plague bacillus has been observed in this country, although cases of this description occur scattered in epizootics of hog cholera with intestinal lesions. Until such an epizootic is observed in this country it is not likely that the question will be thoroughly elucidated as to the rôle of the swine-plague bacilli. It is possible that the swine-plague bacilli are frequently present in the mouth, the air passages or the intestine of healthy hogs, analogous to the frequent presence of the micrococcus of sputum septicaemia and of pneumonia in the mouth of human beings, and that in the mixed infections which we have observed the wide spread diffusion of the swine-plague bacilli is due to secondary invasion following infection with the hog-cholera bacilli. This, however, does not remove the grave significance of the swine-plague bacilli, which certainly cannot be ignored in our studies in this country of the diseases known as hog cholera or swine plague.

While differing in some points from the conclusions reached by the workers on this subject in the Bureau of Animal Industry, we take pleasure in recording the essential harmony of our observations with the facts which they have observed in their painstaking and creditable investigations of this difficult subject as reported since the year 1885.

Through the kindness of Dr. F. S. Billings we have had the opportunity of examining a number of cultures from diseased swine in Nebraska, chiefly direct cultures from the spleen. These in nearly all instances were pure cultures of the hog-cholera bacillus. Much confusion has resulted from Dr. Billings' attempt to identify this organism with that of Schweine-seuche.

We have had the opportunity of examining cultures of Schweine-seuche and also of the Scandinavian swine pest, obtained from the Hygienic Institute in Berlin. The organism in the Schweine-seuche cultures is apparently identical with the swine-plague bacillus which we have isolated. The organism in the swine pest cultures is a different species of bacillus, and appears to resemble closely, if it is not identical with, the hog-cholera bacillus.

We regard it of importance that the future study of swine affected with hog cholera or swine plague should be accompanied with a more thorough bacteriological examination of each case than has hitherto been customary. The mere production of a direct stab culture from one organ, such as the spleen, or the mere inoculation of an animal with material from one organ affords very incomplete and unsatisfactory information. So long as the relations of the two organisms, the hog-cholera bacillus and the swine-plague bacillus, to the diseases of swine are not thoroughly clear, it seems to us necessary to make Esmarch or plate cultures from the blood, the intestine and the principal organs of the body, and also to inoculate animals with material from the lungs, spleen, intestine, etc. The full report of our cases will make clear the value of both of these procedures. A single case thoroughly investigated according to modern bacteriological methods is of more value than many cases in which only stab cultures have been made from one or two organs, or in which reliance is placed solely on the results of inoculating animals. We are also convinced that little reliance can be placed upon the results of experimental inoculations of swine with the suspected organisms of hog cholera and of swine plague in regions where the disease prevails, unless very strict precautions are taken in the selection and care of the experimental animals.

REMARKS ON HOG CHOLERA AND SWINE PLAGUE[1]

(From the Pathological Laboratory of The Johns Hopkins University)

Our observations of hog cholera and swine plague relate mostly to these diseases as they have occurred in Maryland, particularly in the vicinity of Baltimore, since the year 1887, although we have examined cases, specimens, and cultures, also, from various other parts of the country. Our work on this subject began soon after the publication of the discovery of the germs of hog cholera and of swine plague by Salmon and Theobald Smith, and has been carried on independently of that of the Bureau of Animal Industry. Although the publications of this Bureau have, to a large extent, anticipated the publication of our results, nevertheless the conclusions and the manner of their presentation by the Bureau of Animal Industry have been subjected to so much hostile criticism (most of it, in our opinion, uncalled for and unjust), and there has ensued so much confusion as to this whole subject, that it seems important to present additional and independent observations of these epizootic diseases of swine in this country.

Our results are in large measure confirmatory of those reached by the Washington Bureau. As will appear from this paper, there are, however, some points as to which we have arrived at different conclusions from those of the Bureau, and we believe that we have added some new facts of importance. We also claim to have placed upon a firmer basis, by the observance of stricter precautions in guarding against possible error, some of the conclusions reached by the Bureau upon what we regard as insufficient evidence.

Our studies have extended over several years, and have been made upon more than twenty herds of swine in different epizootics. On this occasion we propose to present the more important general results of our investigations, especially as to points which may be considered not wholly settled, and to leave the presentation of our protocols and the full details to a later monograph.

We have adopted the nomenclature of the Bureau of Animal Industry, and understand by the hog-cholera bacillus the microorganism which was first clearly described by Theobald Smith, and which is called by this name in the various reports and publications of the Bureau since their third report for the year 1886 (published in 1887). By hog cholera we understand the disease in swine caused by this bacillus. We employ the name swine-plague bacillus for the microorganism so designated in the reports of the Bureau of Animal Industry since that for 1886, and by swine plague we understand the disease caused by this bacillus.

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[1] William H. Welch and A. W. Clement, V. S. Delivered before the First International Veterinary Congress of America, Chicago, Ill., October 20, 1893. Phila., 1894, 37 p., 8°.

The nomenclature of the epizootic diseases of swine has become so confusing that it is difficult for one not familiar with the various steps in the development of our knowledge of these diseases, and with the varying usage of different writers, to pick his way intelligently through the recent literature of this subject. In each country where hog cholera occurs different names are used for it. In England it is generally called swine fever, and by Klein, also, pneumo-enteritis of swine; in Denmark and Sweden, swine pest (Svinpest) and swine diphtheritis (Svinedephteritis), and in France, pneumo-enteritis of swine (pneumo-entérite du porc), and lately the American name, hog cholera, has also been used in France. Some confusion, particularly in the minds of some German writers, and excusably so, has resulted from the fact that the disease called hog cholera by the Bureau of Animal Industry and by veterinarians in this country, is called by F. Billings swine plague. In German writings the name swine plague seems to be more generally employed to designate the American hog cholera than is the latter name. We venture to suggest that if German writers in this respect would conform to American usage and use the name hog cholera when speaking of the American disease, some confusion would be avoided.

The affection called swine plague by the Bureau of Animal Industry since the report for 1886 (in the report for 1885 this name was given to hog cholera) and by us, was for a time not admitted to exist by F. Billings, and is now accepted by him only as a complication of hog cholera of relatively slight importance. Swine plague seems to be identical with the German Schweine-seuche, although we do not consider this identity, for reasons which will be stated later, as settled beyond all doubt. It has been designated, also, infectious pneumonia of swine, and is regarded by the Bureau of Animal Industry as an infectious pneumo-enteritis. The disentanglement of the two diseases, hog cholera and swine plague and still more of the two microorganisms, the hog-cholera bacillus and the swine-plague bacillus, as regards their relations to each other and to the bacteria isolated from swine epizootics in Europe, and even as to their relation to swine diseases in this country, has been rendered difficult partly in consequence of difficulties inherent in the bacteriological study of these diseases, but largely from imperfections in these bacteriological studies, both in this country and in Europe, and also from ill-considered and confusing writings by more than one contributor to the literature of these subjects.

We do not consider that the names hog cholera and swine plague are either of them particularly fortunate designations, but usage controls nomenclature in medicine as well as in ordinary speech, and we believe that it would add still more to existing confusion were we to invent or adopt other names which are likely to supplant those in ordinary use. Some consensus of opinion is requisite in securing the employment of new terms. We beg to suggest that an authoritative expression of opinion by the United States Veterinary Association as to the best nomenclature of the epizootic diseases of swine would be useful, especially in this matter the Association would act in co-operation with foreign societies and authorities, so as to secure a greater degree of uniformity in international usage. Our understanding of these diseases is now sufficiently complete to justify such an undertaking.

We shall give, in the first place, a brief summary of the more important lesions of hog cholera which we have observed. The possible anatomical lesions of hog cholera are so manifold that a large experience is necessary to render one practically familiar with all of them. Of these lesions, the so-called "buttons" in the intestine are unquestionably the most characteristic. These buttons are elevated, circumscribed, round or oval areas of necrotic inflammation, of firm consistence, of yellowish black, or black color, often presenting an appearance of concentric rings, and involving the mucous and submucous coats, and sometimes all of the coats of the intestine. They are sufficiently familiar to members of this Association.

Scarcely less common, but much less characteristic, than the buttons are diffuse and circumscribed superficial necroses and diffuse and circumscribed diphtheritic inflammation of the intestinal mucosa. Ulcers, haemorrhages, hyperaemia, follicular swellings, and catarrhal inflammation of the intestine are common. The intestinal lesions affect especially the large intestine, being often, although not invariably, most intense near the ileocaecal valve. They may be limited to the large intestine, but it is not rare for the small intestine and stomach to be similarly affected.

Necrotic areas similar to those called "buttons" may occur in various parts of the body besides the intestine and stomach. We have observed them repeatedly on the pharynx, the tonsils, tongue, gums, lips, other parts of the buccal mucosa, the nasal mucous membrane, conjunctivae, gall-bladder and bile ducts (where, indeed, they are common), praeputial sac, vagina, and on various parts of the integument. They are characterized by essentially similar histological appearances in all of these situations—an exudate of fibrin and leucocytes, proliferation of the fixed cells, capillary thrombosis in an early stage, followed by speedy coagulative necrosis, with much nuclear fragmentation of the exudative and tissue cells.

Redness of the skin, particularly of the belly, inside of the thighs, and about the ears, is common, but not constant.

Swelling of the lymphatic glands, especially of the inguinal, mesenteric, retroperitoneal and bronchial, sometimes of all of the lymphatic glands of the body, is common in acute cases, but not constant. This swelling is often accompanied by haemorrhages into the glands and foci of necrosis, sometimes visible to the naked eye, sometimes revealed only by the microscope, these changes giving variegated, mottled appearance to the glands.

Small yellowish, or yellowish white, or reddish foci of necrosis are common in the liver, both macroscopic and microscopic.

The condition of the spleen is variable; it may be unaffected or swollen, firm or soft, and contain fibrous thrombi or, rarely infarcts or haemorrhages.

An interesting change, hitherto overlooked, is the occasional occurrence of hyaline thrombosis of the renal capillaries, both glomerular and intertubular. When this condition is well marked, there are albuminura and even anuria, and it is impossible to force more than a minimal quantity of injecting fluid into the renal vessels. The hyaline stains like fibrin with Weigert's fibrin stain, and is evidently closely allied to fibrin. Sections of such a kidney, stained with Weigert's fibrin stain, look almost as if the bloodvessels had been injected artificially with Berlin blue. This thrombosis may be associated with little alteration of the tubular epithelium, or with or without the thrombosis there may be fatty degeneration of the epithelium, genuine diffuse nephritis, and haemorrhages in the kidney.

Pyelitis, usually haemorrhagic in character, and haemorrhages into the mucous membrane of the bladder, we have repeatedly observed. Blood-clots may be found in the renal pelvis. General fibrinous pleurisy may occur. Diffuse peritonitis we have observed only in hog cholera complicated by the presence of other bacteria.

It is the opinion of the Bureau of Animal Industry that pneumonia is not a lesion which belongs to uncomplicated hog cholera, although some bronchopneumonia is admitted to be sometimes present. Various types of pneumonia are found in many pigs dead of hog cholera. In the great majority of our cases strongyles were present in the bronchi, and foci of bronchopneumonia could be referred to their presence. There were, however, observed in many cases larger or smaller areas of hepatization, often partly necrotic, and of types which cannot be referred to the presence of strongyles. In a large proportion of these cases the swine-plague bacillus was present, with or without coincidence of the hog-cholera bacillus, in the lungs, and as the swine-plague bacillus can be shown experimentally to be capable of producing pneumonia, these cases cannot be adduced in support of the view that the hog-cholera bacillus alone may cause pneumonia. We have, however, observed some cases of pneumonia not referable to strongyles, in which the hog-cholera bacillus was present alone in the lungs, and as we possess experimental evidence, as will be explained later, that the hog-cholera bacillus may produce pneumonia in swine, we are of the opinion that pneumonia may be a lesion of uncomplicated hog cholera. This pneumonia may be associated with fibrinous pleurisy, also referable to the hog-cholera bacillus.

Fatty degeneration of the heart may occur in hog cholera. Acute and chronic endocarditis and myocarditis are rare lesions which we have observed.

In not a few acute, and in some chronic cases, ecchymoses are so numerous in the skin, mucous and serous membranes, and in the viscera, that a haemorrhagic type of the disease may properly be distinguished. Even diffuse haemorrhages may occur, particularly in the connective tissue around the kidney. Acute cases may occur of a septicaemic type without intestinal lesions.

Although a distinction between acute and chronic hog cholera is in many cases useful and appropriate, there are so many transitional types of the disease that it should be understood that there are no sharp dividing lines between acute and chronic cases.

We have had opportunity to study the process of repair of the intestinal lesions in pigs which have partly or wholly recovered from natural and experimental hog cholera, but we shall not take this opportunity to describe these interesting reparative changes.

The greater our experience in the bacteriological study of hog cholera became, the more convinced were we of the importance of completeness in the bacteriological examination of each case. To obtain thoroughly trustworthy and satisfactory results we consider it important in each case to make cover-slip preparations and cultures from the various organs of the body, especially from the lungs, spleen, heart's blood, kidney, liver, lymphatic glands (particularly the mesenteric), intestinal lesions and inflammatory exudates elsewhere if present, and also to inoculate mice or rabbits with bits of these organs and lesions. Such an examination takes much time, but a few cases carefully studied in this thorough manner are worth more than many cases imperfectly studied.

Without such complete examination it will often happen that one cannot be sure that other microorganisms, particularly the swine-plague bacillus, were not present in some of the internal viscera, together with the hog-cholera bacillus. The hog-cholera bacillus may be present in such small number and so limited in distribution that it is likely to be overlooked unless all of the parts named are examined both by cultures and by inoculation of animals. It is often necessary to inoculate the culture tubes with tolerably large fragments of the spleen and other parts in order to detect the presence of hog-cholera bacilli, so few may these be. The colonies of the hog-cholera bacillus are generally distinguishable from those of the swine-plague bacillus by their larger size and coarser, more opaque, grayish white growth; but sometimes, especially in roll and plate cultures crowded with colonies, these differences are so little marked and each of these varieties of colony is in itself so little characteristic that it is particularly important to secure the aid of inoculation of animals with parts of organs to differentiate these organisms and to recognize the presence of one or both. If both swine-plague bacilli and hog-cholera bacilli are present in the part examined, rabbits and mice inoculated with this part will die of swine plague, and if reliance were placed solely upon the animal experiment the hog-cholera bacillus, if present, would be overlooked.

The statement is made in publications of the Bureau of Animal Industry, that if the hog-cholera bacillus is present, it will be found invariably in the spleen.[2] In the cases in which we have demonstrated the presence of the hog-cholera bacillus we have very rarely failed to find it in the spleen. Still we have notes of a few cases in which both the inoculation of animals and of culture media with good sized bits of the spleen has yielded negative results, and the hog-cholera bacillus has been found in other parts, so that we must dissent from this statement. We have found the hog-cholera bacillus in the intestine when it has been absent from the spleen.

When a mixture of pathogenic organisms has been present we have been helped out occasionally, especially in examining the intestine, by making roll or plate cultures from the neighborhood of the seat of inoculation in rabbits. By this means we have detected both the swine-plague bacillus and the hog-cholera bacillus together in the intestine, when otherwise we should have failed to find the latter organism.

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[2] Theobald Smith: "Special Report on the Cause and Prevention of Swine Plague," pp. 44, 103, Washington, 1891, and other places. It should be said, however, that Dr. Smith admits the possibility of the localization of the hog-cholera bacillus in the intestine, when it is absent from internal organs, but he has not published observations demonstrating this fact. He says, op. cit., p. 103: "The difficulty of examining the intestines for pathogenic bacteria and the amount of labor involved is very great, and hence for want of time and sufficient assistance this part of the work has been set aside in these investigations, and the attention centred on the lungs and other internal organs." Notwithstanding this admission, we do not think that he always recognizes sufficiently in his publications the possibility that some of the cases with intestinal lesions which he regards as pure swine plague may have been combined swine plague and hog cholera, with localization of the hog-cholera bacilli in the intestine, nor does he appear to know that rarely hog-cholera bacilli may not be found in the spleen, even when demonstrable in other internal organs.

One of us (Welch) has described in an article published in 1889[1] the principal characters of the hog-cholera bacillus and of the swine-plague bacillus, and it is not necessary to repeat here this description, which was essentially confirmatory of the results obtained in the Bureau of Animal Industry.

We wish, however, in this connection, to express our conviction that the organism described by Selander in 1890, and probably also that by Metchnikoff later, as the hog-cholera (or swine-pest) bacillus is not identical with the genuine hog-cholera bacillus as we know it in this country. This point needs especial emphasis, as the Metchnikoff hog-cholera bacillus seems to have been accepted without question as the genuine hog-cholera bacillus, and the already existing great confusion as regards this organism is likely to become almost inextricable unless this error be corrected.

Our present criticism refers only to the organism described by Selander in 1890, in the "Annales de l'Institut Pasteur," and not to that described by him as the bacterium of swine pest in the "Centralblatt für Bakteriologie" in 1888. By experiments conducted in Pasteur's laboratory under Roux, Selander, starting with a culture of the swine-pest bacillus of 1888, which killed rabbits in three to seven days, claimed by a special procedure to enhance the virulence of this bacillus to a point at which it killed pigeons, which are not very susceptible to the hog-cholera bacillus, in ten to twelve hours, and rabbits in twelve to fifteen hours by subcutaneous injection of small doses, and in five hours by intravenous injection. The procedure consisted essentially in inoculating in series rabbits with the crushed spleens in bouillon of rabbits dead of inoculation with the bacillus, the spleen of one rabbit being used to inoculate the next, and so on. He found that the fourth rabit in this series died in fourteen hours. The virulence was still further enhanced by inoculating pigeons in series.

Selander's statements were so much at variance with our experience that we have repeated his experiments exactly according to his directions. We have carried the series to over twenty rabbits without obtaining any noticeable diminution in the duration of life of the inoculated rabbits. At the end, as well as at the beginning, of the series the rabbits died in four to six days after inoculation.

The hog-cholera bacillus is most tenacious of its special characters. Original variations in virulence, and to some extent in other properties, occur, but with one and the same bacillus we have not succeeded by successive inoculation of animals in producing very marked changes in the degree of virulence, nor have we ever met a kind of hog-cholera bacillus which, by subcutaneous inoculation of ordinary doses, regularly killed rabbits in less than three days, although occasionally rabbits may die in less than that time.

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[1] William H. Welch: "Preliminary Report of Investigations Concerning the Causation of Hog Cholera," The Johns Hopkins Hospital Bulletin, December, 1889.

Dr. Selander, through the friendly mediation of Prof. Salomonsen, of Copenhagen, kindly supplied one of us (Welch) in August, 1890, with a sealed tube[4] of the blood of a pigeon dead of inoculation with his bacillus, and also with an hermetically sealed agar culture of the same bacillus of exalted virulence. We beg to express our thanks to both Dr. Selander and Prof. Salomonsen. These tubes were received in Berlin, and were brought to this country by one of us in the same package with cultures of Schweineseuche, swine-pest, swine-fever, and other bacteria. They were opened and examined early in October.

Selander's tubes contained a short, oval bacillus, resembling the bacteria of the haemorrhagic septicaemia group. The organisms in both tubes were dead, neither cultures therefrom nor inoculation of rabbits or mice yielding positive results. The cultures of various other organisms conveyed in the same package from Berlin were all alive. The media in Selander's tubes were not at all dried down. Unless some unknown injurious agency acted upon these cultures of Selander's bacillus before they were handed to us by Prof. Salomonsen, the fact that the cultures were dead excludes, we believe, the presence in them of the hog-cholera bacillus, which survives at least two years in agar culture protected from desiccation. Selander, however, believes that his bacillus with exalted virulence has become less resistant than the hog-cholera bacillus of ordinary virulence; but in view of our experience in repeating Selander's experiments to heighten virulence, we believe that the bacillus which he obtained after serial inoculations of rabbits and pigeons was not derived from the hog-cholera (swine-pest) bacillus, but was some different species of microorganism.

We should suspect that possibly in the course of his experiments there developed a case of rabbit septicaemia, and that the subsequent work was done with a culture from such a source, were it not for his statement that the subcutaneous, intravenous, or alimentary inoculation of swine with his bacillus with heightened virulence produced diphtheritic enteritis, a lesion which is not known to be caused in swine by the bacillus of rabbit septicaemia. Microscopically, the bacteria in Selander's cultures given us were decidedly smaller than the hog-cholera bacillus. Many bacilli of the haemorrhagic septicaemia group die in a few days or weeks in artificial culture. In fact, we have had some difficulty in obtaining from Europe living cultures of the Schweine-seuche bacillus which belongs to this group. Whatever this organism of Selander may have been, we feel convinced that it was not our hog-cholera bacillus, and we base this conclusion upon the results of his experiments in Pasteur's Institute, as published, and upon our experience with the cultures with which he kindly supplied us.

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[4] One of these tubes was dated either April 28, 1890, or July 28, 1890; we could not positively make out which. Assuming that it was the earlier date, the culture, when examined, would have been less than six months old. The other tube was undated. As the dates of Selander's experiments in Pasteur's Institute, as given in his article, run from March to June, 1890, the cultures could hardly have been at the utmost more than seven or eight months old, and were probably, as appears from the date mentioned above, less than that age.

We are unable, moreover, to reconcile with the properties of the genuine hog-cholera bacillus the description given by Metchnikoff of the so-called microbe of hog cholera with which he performed his experiments on immunity, described in the "Annales de l'Institut Pasteur," May, 1892. He says that he has obtained the same results as those of Selander. He describes his hog-cholera microbe as most pronouncedly pleomorphic, growing in short rods, long threads, and veritable cocci, sometimes as a streptococcus and killing rabbits in a few hours. He gives it the name *Coccobacillus suis.* This name and this description are singularly inappropriate for the genuine hog-cholera bacillus, which is no more pleomorphic than the typhoid bacillus. Unlike the swine-plague bacillus, it does not grow in forms suggesting cocci. Metchnikoff states that the blood serum of the rabbit is not germicidal for his hog-cholera microbe. In our experience the normal rabbit's serum is germicidal for the hog-cholera bacillus to about the same extent that it is for the typhoid bacillus. We believe Metchnikoff's hog-cholera microbe to be one of the haemorrhagic septicaemia bacilli, possibly the swine-plague or Schweine-seuche bacillus.

In many German publications the hog-cholera bacillus is placed under the group of bacteria belonging to the so-called haemorrhagic septicaemias. Elaborate studies have been made in German laboratories, showing that the hog-cholera bacillus is not identical with the Schweine-seuche bacillus, which is a member of the haemorrhagic septicaemia group. There never was any good reason to suppose that it could be identical with the latter bacillus, and the amount of labor given to repeatedly demonstrating this evident fact, sufficiently familiar to us since 1888, has been a source of surprise to American investigators (with the possible exception of F. Billings) who were acquainted with the hog-cholera bacillus on the one hand and the various bacteria of the haemorrhagic septicaemia group on the other hand. We consider that the hog-cholera bacillus bears more resemblance to the typhoid and colon groups of bacteria (although nothing would be gained by classifying it with either group) than to the haemorrhagic septicaemia group, and that it is misleading to place it in the latter group, where the swine-plague bacillus, the Schweine-seuche bacillus, the Wild-seuche bacillus, etc., belong.

As regards the distribution and recognition of the hog-cholera bacillus in the natural disease in swine, there are certain points which we wish to emphasize. Although in ordinary cases of hog cholera there is no difficulty in recognizing the presence of the hog-cholera bacillus, cases occur in which the determination of the presence of this bacillus is difficult, and there are chronic cases with typical lesions of hog cholera, and cases even with apparently fresh lesions, in which the most complete bacteriological examination fails to reveal the presence of this microorganism.

In one of our cases we demonstrated hog-cholera bacilli in abundance in the intestine, which was the seat of rather superficial small buttons and of fresh diphtheritis, and found only a single colony of the hog-cholera bacillus in the roll culture from the liver, and a very few colonies in the culture from the peritoneum. Cultures inoculated with abundant material from the spleen, blood, lungs, and kidney contained no colonies of this bacillus, and a rabbit inoculated with the spleen survived. This case shows that the bacteriological examination of the intestine in hog cholera, difficult as it is, should not be neglected, and that without such examination the presence of hog-cholera bacilli may be overlooked. In several similar cases with intestinal lesions typical of hog cholera, swine-plague bacilli were present in the lungs and likewise sometimes in other parts. The danger of mistaking such cases for pure swine-plague infection, so far as can be determined by bacteriological examination, is apparent.

We have evidence that the hog-cholera bacillus may have entirely disappeared from the body, so far as can be determined by cultures and inoculation of animals from the various organs of the body, including the intestine, at a period when characteristic intestinal lesions are still present. This evidence rests upon the study of herds in which we have found pigs dead of hog cholera with hog-cholera bacilli, and later have examined, without finding these bacilli, pigs from the same herd, with similar intestinal lesions—some evidently undergoing repair, others still typical buttons.

Pigs in which the intestinal lesions of hog cholera persist after the disappearance of the hog-cholera bacilli may be infected also with swine plague. We interpret thus cases in which the most typical intestinal lesions of hog cholera—that is, perfectly characteristic buttons—are present, and still the bacteriological examination shows the presence of only swine-plague bacilli. We have observed whole herds in which the affection was of this character. These buttons we regard as characteristic of hog cholera—as much so as typhoid ulcers are of typhoid fever. We possess convincing experimental proof that these buttons are caused by the hog-cholera bacillus, and there is no evidence that the swine-plague bacillus or any other species of microorganism is capable of producing them.[5] In the present state of our knowledge we consider that whenever typical buttons are present the inference is justifiable that the hog-cholera bacillus has been active in the case, whether or not it be demonstrable at the time of autopsy. There is no difficulty in understanding how such necrotic lesions, when extensive, may lead to the death of the pig, even after the specific germ has died out. In most, but not all, of these fatal chronic cases with lesions characteristic of hog cholera, but without the hog-cholera bacillus, there is concurrent or secondary infection with another microorganism, most frequently with the swine-plague bacillus, and the death of the animal may be attributed in part or wholly to this other infection.

Characteristic buttons are by no means present in all cases of hog cholera—not even in all chronic cases. We possess evidence similiar to that already mentioned, based upon the examination of previous or concurrent cases of hog cholera in the herd, that pigs may die with forms of intestinal necrosis which cannot properly be called buttons, and even with simple intestinal diphtheritis after the hog-cholera bacilli have disappeared, or at least are no longer demonstrable by culture or inoculation of animals, although they had been demonstrated previously in cases with similar lesions in the same herd. Such observations with negative bacteriological results might be interpreted as opposed to the acceptance of the hog-cholera bacillus as the cause of hog cholera, were it not for the conclusive experimental demonstration of the causative rôle of this bacillus.

In view of the facts which have been mentioned, it is clear that great caution should be exercised in the interpretation of cases in which the bacteriological examination reveals only the swine-plague bacillus, although the intestinal lesions of hog cholera are present. Until there is experimental evidence that the swine-plague bacillus can produce such lesions, it is not warrantable to refer them to the presence of this organism.

In a number of cases we have isolated hog-cholera bacilli of varying degrees of attenuation of virulence. The attenuated form most frequently met is one which fails to kill rabbits by subcutaneous inoculation, but is fatal after intravenous inoculation with the characteristic lesions produced by the virulent bacillus, but with a greater tendency by this method of inoculation to cause intestinal haemorrhages, necrosis, and diphtheritis in rabbits. Similar observations have been made by Theobald Smith. These intestinal lesions in rabbits and guinea pigs are not, however, extremely infrequent after intravenous, and they may occur after subcutaneous inoculation, and often after ingestion of virulent hog-cholera bacilli. We have also met bacilli with all essential morphological and cultural characters of the hog-cholera bacillus, but incapable of killing either rabbits or mice, at least in ordinary doses. We have succeeded in causing intestinal diphtheritis with necroses by feeding swine cultures of partly attenuated hog-cholera bacilli, although the pigs have usually recovered. These observations are of importance with reference to the question of identity of the hog-cholera bacillus and the swine-pest bacillus, as will be explained later.

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[5] An exception would be made to this statement if the recently published view of Bang were confirmed, that still a third bacterium, which he calls the necrosis bacillus, is concerned in the natural disease designated hog cholera or swine pest. The deeper necroses, that is, the buttons, he attributes to the invasion of this necrosis bacillus after the way has been prepared for it by a superficial croupous inflammation set up by the hog-cholera bacillus. Our experiments, however, show that the hog-cholera bacillus may cause these buttons, and we cannot, therefore, accept with the present evidence a second microorganism as the cause of such peculiar and typical lesions. We are acquainted with Bang's article only by the abstract in the Centralblatt für Bakteriologie, 1893, Bd. XIII, 203.

As we have found in the same pig both virulent and attenuated hog-cholera bacilli, it is probable that the hog-cholera bacillus may suffer loss of virulence in the body of swine affected with hog cholera. It is not necessary to assume that the degree of virulence found at autopsy is identical with that possessed by the bacillus at the time of invasion of the animal.

In several instances the subcutaneous inoculation of rabbits with necrotic intestinal buttons has not proven fatal, even when the inoculation with the spleen and other organs of the same pig has caused fatal hog cholera in rabbits. This would indicate that the hog-cholera bacilli were no longer present in the buttons, or, if present, were, in contrast with those in the spleen and other internal organs, so weakened in virulence as not to be fatal to rabbits by subcutaneous inoculation, or that the mixture with intestinal bacteria prevented the usual manifestation of their pathogenic activity. The last supposition we consider improbable, as often, indeed usually, the inoculation of rabbits with the fresh intestinal lesions of uncomplicated hog cholera causes death, with the usual lesions and presence of hog-cholera bacilli in the rabbit. In the combined infection with hog cholera and swine plague the inoculation of rabbits with pieces of the intestine is likely to produce swine plague.

In our experience there has been considerable similarity in the results of the bacteriological examination of the different pigs of the same herd, whether affected with pure hog cholera or with hog cholera combined with swine plague; but there are so many exceptions to this that it is hardly proper to set up any rule on this point.

Often in hog cholera there may be invasion of other bacteria than that mentioned. The most frequent of these secondary invaders (excluding the swine-plague bacillus) has been in our experience *Bacillus coli communis*, which may, without sufficiently careful examination, be mistaken for the hog-cholera bacillus, which morphologically, and even in many of its cultural properties, is not so unlike the colon bacillus. As has been shown by one of us (Welch[6]), intestinal lesions such as those present in hog cholera favor the invasion into internal organs of *Bacillus coli communis*.

We will now consider briefly the experimental evidence that the hog-cholera bacillus is really the cause of hog cholera. Although the bacteriological study of the natural disease speaks decidedly for the hog-cholera bacillus as the cause of the disease, there are such difficulties in this study, and so many complicating factors, that a reasonable skepticism is warranted until the conclusive experimental demonstration is brought that the hog-cholera bacillus, when inoculated in pure culture into healthy swine, is capable of reproducing the disease as it is observed under natural conditions. Above all, it should be demanded that the typical button-like lesions are reproduced. The production of simple intestinal diphtheritis, with which previous experimenters seem to have been content, is a lesion which may be caused experimentally in animals by various microorganisms, as well as by irritant substances, and cannot be considered a satisfactory reproduction of the natural disease in its most characteristic feature, although it is true that the natural disease occurs with diffuse intestinal diphtheritis as the only intestinal lesion.

It is not clear to us that other investigators have succeeded under proper precautions in reproducing these buttons experimentally, and this gap in the experimental evidence we are able to fill.

We shall not discuss here our experiments upon rabbits, mice, guinea pigs, and pigeons, interesting as are the lesions produced in these animals by the hog-cholera bacillus.

We have adopted every precaution to avoid error in the experiments on swine, and we consider that without such precaution experiments on swine with the hog-cholera bacillus in regions where the natural disease prevails are of little value. We have purchased the experimental pigs from places where disease was not present and had not been known to occur, and from persons who raised their own pigs, not purchasing from others. We soon found that the dangers of accidental infection with hog cholera in the Pathological Laboratory of the Johns Hopkins University, where we made autopsies on the natural disease, were such that we abandoned such experiments there, and erected pens or kept boxes in clean stables and other places where there was no possibility of infection of the locality, and we frequently shifted from place to place in making the series of experiments. Every precaution was taken to guard against accidental infection in feeding or in other ways, and in this we were usually successful. We consider it necessary to reserve as control animals pigs selected from the same lot used for the experiment, and to keep the control pigs under the same external conditions and in the same locality, but, of course, in separate boxes or pens, with the animals experimented upon. Our early experience in buying pigs brought by rail or boat to the city, and in not being minutely careful to avoid accidental infection, led us soon to realize the importance of these painstaking precautions, tedious and expensive as they often were. We felt obliged to reject not a few experiments in which the control pigs were found affected; but we have records of a large number of experiments in which the control pigs were healthy, the utmost caution was observed, and which we believe will stand the most rigid scrutiny.

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[6] Welch: *Bacillus coli communis;* the Conditions of its Invasion of the Human Body and its Pathogenic Properties, The Medical News, December 12, 1891.

Time will not permit on this occasion to give more than a general statement of our results, the details of which we expect to publish later.

We have succeeded, by inoculation of pure cultures of the hog-cholera bacillus, in reproducing experimentally in pigs every lesion of the natural disease, including necrotic buttons in the intestine and in all of the other situations mentioned above in describing the situation of such necroses, pneumonia, hepatic necroses, hyaline thrombosis of renal vessels, ecchymoses, etc.

It is hardly necessary to say that our experimental cases were subjected to careful bacteriological examination, and that in the successful cases the hog-cholera bacilli were demonstrated in pure culture.

Subcutaneous inoculation of swine with cultures of the hog-cholera bacillus usually produces a local inflammation with necrosis and sequestration of the inflamed tissues in which the virulent bacilli may persist for months. The animals nearly always recover. Rarely in our experience this mode of inoculation produces general fatal infection with intestinal lesions, such as are observed after feeding and other modes of inoculation.

Feeding cultures of the hog-cholera bacillus produces with great regularity intestinal diphtheritis and general infection without pneumonia. Usually the animal dies after a few days up to two or three weeks, but it may recover; and such cases of recovery afford opportunity to study the reparative process in the intesine. By simply rubbing the lips of pigs with potato cultures we have produced the disease in fatal form. With or without diphtheritic inflammation of the stomach and small intestine we have usually found in these cases an extensive superficial necrosis and diphtheritic inflammation of the mucous membrane, sometimes also reaching the submucous coat, of the large intestine, most diffuse near the ileocaecal valve and occurring in the lower part of the colon in the form of more or less circumscribed patches of necrosis and diphtheritis. Some of these circumscribed necrotic patches bear some resemblance to the buttons; but the regular, typical, round, firm, elevated buttons we have been more successful in reproducing by intravenous and intratracheal inoculations.

Intravenous inoculations produce usually rapidly fatal forms of infection, sometimes of distinctly haemorrhagic and septicaemic type, usually with at least some intestinal diphtheritis, but occasionally without diphtheritis. We have been so fortunate in a few cases after intravenous injection of very small doses (0.05 c. c. bouillon culture and less) in keeping the animal alive for a longer time and in finding at autopsy typical buttons in the large intestine.

These cases, together with similar ones, as regards intestinal lesions after intratracheal inoculation, have enabled us to study different stages in the formation of these buttons, and we are able to say that at least some of the buttons begin as circumscribed nodular inflammatory masses in the submucosa, with, at an early stage, an overlying intact mucosa.[7] We have evidence also of the possibility of the buttons beginning by necrosis and inflammation of the mucous membrane and secondary involvement of the submucosa.

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[7] The demonstration by us that the buttons may start by nodular foci of inflammation in the submucosa, and that there may be coagulative necrosis in these submucous nodules at a period before the mucous menbrane is necrotic, is a strong argument in opposition to Bang's view, mentioned in a previous foot-note, that these deep necroses are produced by his necrosis bacillus and that only the superficial diphtheritic or croupous inflammation is caused by the hog-cholera bacillus, this latter organism simply preparing the way for the penetration of the necrosis bacillus which he finds to be present now and then in the intestine of healthy pigs. We have found the hog-cholera bacillus in the submucous nodules, and sometimes apparently without admixture with other micro-organisms. It must be admitted, however, that it is difficult to succeed in producing typical buttons by inoculating healthy swine with the hog-cholera bacillus.

That the hog-cholera bacillus is capable of producing extensive necroses cannot be doubted, as the result of experiments on rabbits, mice, guinea pigs, and on swine proves. If anyone wishes to convince himself as to the power of the hog-cholera bacillus to produce extensive and deep local necroses, let him inoculate the organism into the breast muscle of a pigeon. The foci of hepatic necroses found so regularly in rabbits and mice inoculated with the hog-cholera bacillus may be caused also, although less frequently, by the swine-plague bacillus and by many other bacterial species. Bang's view that the necroses in the pneumonia caused by the swine-plague germ (he does not seem to recognize this lesion as referable to the hog-cholera bacillus) are due also to the necrosis bacillus, we also cannot accept, as necrotic pneumonias can readily be produced by intratracheal or intrapulmonary inoculation of the swine-plague bacillus, and at autopsy only the swine-plague bacilli be present.

Intrapulmonary inoculation through the chest wall often produces only circumscribed inflammation and sequestration of a limited area of lung at the point of inoculation, but it may cause diffuse hepatization, with general infection and intestinal lesions.

The most successful reproduction of characteristic pneumonia, associated with typical intestinal lesions, we have obtained by intratracheal inoculation of bouillon cultures in moderate doses. By this method we have caused diffuse hepatization of one-third and more of both lungs. The hepatization was variegated, red, gray, white, with areas of coagulation necrosis, with exudation of fibrin and leucocytes, with thrombi in the vessels, and with interstitial exudate; in a word, pneumonia resembling forms occurring in the natural disease. In some of these cases there were no strongyles in the bronchi. The hog-cholera bacilli were present in large number and in pure culture, positively without swine-plague bacilli in the lungs and other internal organs. When, as in some of these cases, there were intestinal buttons, hepatic necroses, hyaline in the kidneys, the reproduction of the natural disease was complete.

We consider, therefore, that the evidence is now complete, more so than in any previous experiments, that the hog-cholera bacillus is the cause of hog cholera in swine. That cases of long-standing hog cholera may terminate fatally at a stage so late that the characteristic bacilli have disappeared cannot be urged in opposition to this conclusion, as we have examples enough of the extinction of pathogenic germs from lesions which they have primarily produced; and as an example particularly pertinent, we cite typhoid fever in its late stage where also death may occur from secondary infection. That many of these fatal cases of hog cholera without hog-cholera bacilli are examples of mixed and secondary infection has already been mentioned. Whenever we find the typical button-like lesions of the intestine which are peculiar to hog cholera, we consider that the case is or has been one of hog cholera whether or not at the time of autopsy living hog-cholera bacilli can be demonstrated in these lesions or elsewhere in the body.

Is the hog-cholera bacillus identical with any of the bacteria which have been found in Europe in diseases of swine? The only bacteria which come into question are the bacillus of swine fever or pneumo-enteritis in England, the bacillus of Scandinavian swine pest, and that of the French pneumo-enteritis of swine. There is not the slightest ground for supposing that the activity motile hog-cholera bacillus, with its peculiar reaction in milk cultures, its visible growth on potato, its characteristic effects on animals, has anything to do with the smaller, non-motile Schweine-seuche bacillus growing invisibly or not at all on potato, without distinctive reaction in milk cultures, and possessed of different pathogenic properties on animals.[*] The latter bacillus belongs to the haemorrhagic septicaemic group, a widely distributed group, established under this name by Hueppe, for which we possess very unsatisfactory means of differentiation of its members from each other. The hog-cholera bacillus, in our judgment, should not be ranked with the members of this group.

As was stated for the first time by one of us (Welch), as the result of a comparison of cultures, in an article published in 1889, F. Billings' swine-plague bacillus is identical with the genuine hog-cholera bacillus. Without mention and probably without knowledge of our work, Frosch and other German investigators later came to the same conclusion. Billings' designation of swine-plague bacillus for the hog-cholera bacillus has not gained currency in this country, whereas the name swine-plague bacillus has been introduced by the Bureau of Animal Industry for a totally different species of bacterium belonging to the haemorrhagic septicaemic group. On this occasion we beg to express our thanks to F. Billings for the numerous cultures which he has sent us.

The anatomical description of the disease swine fever (Klein's pneumo-enteritis), the Scandinavian swine pest, and at least some of the cases of the French pneumo-enteritis of swine, leaves no doubt of the pathological identity of the disease known by these names with the American hog cholera.

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[*] That so many German investigators should have thought is necessary to devote so much time and work to proving over and over again that the hog-cholera bacillus is not identical with the Schweine-seuche bacillus is due to the confusion introduced into this whole subject by the writings of F. Billings. The description of the hog-cholera bacillus in the publications of the Bureau of Animal Industry and in the preliminary report by Welch, 1889, rendered it impossible to confound the hog-cholera bacillus with the Schweine-seuche bacillus. The organisms which really need comparison are the hog-cholera bacillus and the swine-pest bacillus on the one hand, and the swine-plague bacillus and the Schweine-seuche bacillus on the other hand. It is somewhat remarkable that in an article from Baumgarten's laboratory by Raccuglia, entitled "Comparative Experimental Investigations concerning the Bacteria of the German (Löffler-Schütz) Schweine-seuche, the American Swine Plague, and the Danish Swine Pest" (by American swine plague is meant hog cholera), the only bacteria compared by personal experimental work are the Schweine-seuche bacterium and the hog-cholera bacillus, and that no mention is made of personal observations with swine-pest cultures, although such would be expected from the title of the article, and that in the subsequent article in the same volume, by Afanassieff, the only personal observation of the swine-pest bacillus recorded is that it grows, like the hog-cholera bacillus, on potato (Arb. a. d. Gebiete d. path. Anat. herausg. von Baumgarten, Bd. I., 1892). Both of these articles are valuable and careful bacteriological studies.

One of us (Welch) has had the opportunity of examining specimens from cases of English swine fever and Scandinavian swine pest, and has no hesitation in pronouncing these diseases identical, so far as the lesions are concerned, with our hog cholera.

The description originally given by Klein[9] of the bacillus which he then considered to be the cause of the disease in England cannot be made to apply to the hog-cholera bacillus, as his bacillus was said to form spores and to resemble the hay bacillus.

In 1890 Dr. Klein was so kind as to give one of us (Welch) a culture of the bacillus which he had isolated from cases of English swine fever, and which he then regarded as the cause of swine fever. Through the kindness of Professor Brown, Principal of the Royal Veterinary College of London, and of Mr. Banham, of Cambridge, England, one of us (Welch) was permitted to study pathologically and bacteriologically cases of swine fever occurring near Cambridge, England. Professor Klein's culture and the bacillus isolated from the English cases of swine fever proved to be in all respects, including the degree of virulence, identical with our hog-cholera bacillus. A careful comparison of the English cultures with those of our hog-cholera bacillus was made, as regards morphology, cultural behavior, and pathogenic effects, and no difference was found. Klein's present culture of the swine-fever bacillus, therefore, can hardly be the same as that which he originally described as like that of the hay bacillus, but it is the genuine hog-cholera bacillus, which, of course, forms no spores and does not resemble the hay bacillus.

The bacillus originally cultivated by Bang from the Scandinavian swine pest and described by Selander,[10] and, later, studied by other investigators,[11] is doubtless the hog-cholera bacillus, although it would appear to have been observed oftener in an attenuated form than has been our experience with the American hog-cholera bacillus. We have already expressed our opinion that some error crept into Selander's work with this bacillus in Pasteur's Institute, so that the bacillus which he obtained from his experiments there, and which he supposed to be the swine-pest bacillus of highly exalted virulence, was in reality a different bacterium. We at least feel justified in calling for a repetition by others of these experiments of Selander, with the undoubted swine-pest or hog-cholera bacillus, and for an independent examination of cultures of Selander's Paris bacterium, as well as of Metchnikoff's so-called hog-cholera microbe.

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[9] Klein: Virchow's Archiv, Bd. XCV., 1884.

[10] Selander: Centralbl. f. Bakter., 1888, Bd. III. p. 361. This work was done under Gaffky, in the Reichsgesundheitsamt in Berlin.

[11] Jensen: Abstract in Baumgarten's Jahresbericht, 1889, p. 177. (Refers to Bang's investigations.) Welch: *Loc. cit.*, 1889. Frosch: Zeitschrift f. Hygiene, 1890 Bd. IX. Caneva: Centralbl. f. Bakter., 1891, Bd. IX. p. 557. Bunzl-Federn: *Ibid.*, 1891, Bd. IX. 787, and Arch. f. Hygiene, 1891, Bd. XII. Afanassieff: *Op. cit.*, 1892, p. 263. Bang: *Loc. cit.*, 1892-93.

In Selander's original description of the swine-pest bacillus, he speaks of its growing upon potato like the typhoid bacillus. Frosch, who worked with cultures from the same source, was unable to confirm this.[12] As a matter of fact, the appearance of the growth upon potato of the hog-cholera bacillus is somewhat variable, and exceptionally its growth may be scarcely visible or even invisible upon potato, so that we are inclined to the view that Selander did not make a large number of observations of potato cultures of this organism, and happened to observe the exceptional typhoid-like growth. In our preliminary report of 1889 we stated, from personal comparison of cultures, the probable identity of the hog-cholera and swine-pest bacilli.

We were so fortunate as to be supplied again, in 1890, with a culture of the swine-pest bacillus from the Hygienic Institute in Berlin. This was stated to be from the original Bang-Selander stock. Morphologically and in cultures, notably in milk cultures and on potato, we were unable to detect any significant difference between this culture and the hog-cholera cultures. The swine-pest bacillus grew on media somewhat more luxuriantly than the hog-cholera bacillus. Although pathogenic for mice, the swine-pest culture, however, failed to kill rabbits by subcutaneous inoculation, although by intravenous inoculation it was fatal with the same lesions and distribution of the bacteria as in the case of our hog-cholera bacillus. As we have obtained from the hog cholera in this country a bacillus in all respects identical with this swine-pest bacillus, and which we regard as a somewhat attenuated form of the hog-cholera bacillus, we agree with those who have found the Scandinavian swine-pest bacillus to be identical with our hog-cholera bacillus. It remains to be determined by larger experience whether the attenuated bacillus is more common in the Scandinavian cases than in the American cases. It would appear from Bang's latest study of the subject, that the bacillus isolated from chronic cases of Scandinavian swine pest is of decidedly attenuated virulence. Bang found his swine-pest bacillus from the epizootic of 1887 identical with our hog-cholera bacillus.

The culture of the swine-pest bacillus brought to the Bureau of Animal Industry in Washington by Lundgren in 1888 was stated to be devoid of virulence for mice, rabbits, and pigs. It is left undecided by Salmon and Smith whether this was an attenuated culture of the hog-cholera, bacillus, or, as suggested by Lundgren, some other bacillus brought to this country by mistake for the genuine swine-pest germ.

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[12] *Loc. cit.* It should be noted that according to Jensen's article (already cited), published in 1889, Bang distinctly denies the accuracy of this statement of Selander, and says that his swine-pest bacillus is in all respects identical with Salmon's hog-cholera bacillus. Of the subsequent investigators, only Caneva finds this typhoid-like growth of the swine-pest bacillus.

Frosch (1890) concludes that Selander's swine-pest bacillus is identical with the American hog-cholera bacillus, but the only mention which he makes of inoculation of animals with the former is the statement that it possessed less virulence for guinea pigs than was found by Selander, and less than the American hog-cholera bacillus. Selander in his original brief communication (1888) found the swine-pest bacillus virulent for mice, rabbits, and guinea pigs, both by subcutaneous inoculation and by feeding.

Caneva found that the swine-pest bacillus (Selander) was closely allied to the American hog-cholera bacillus (Salmon), but differed from it by typhoid-like growth on potato and by absence of pathogenic effect when inoculated subcutaneously in rabbits. As we have already stated, we, as well as others, found no difference between the potato growth of swine pest and of hog cholera, and that although the swine-pest culture in our possession was not virulent by subcutaneous inoculation of rabbits, it produced by intravenous inoculation the same effect as the hog-cholera bacillus, with the same tendency to cause intestinal lesions in rabbits that was noted with our attenuated hog-cholera bacillus more frequently than with the virulent bacillus. The usual extensive necroses in the liver and the occasional formation of hyaline in the renal vessels were produced by this swine-pest germ as well as by the hog-cholera bacillus.

The only statement made by Afanassieff as to his study of the swine-pest bacillus is that it grows on potato like the hog-cholera bacillus. This leads Baumgarten to remark in a footnote that by this observation of Afanassieff (it had already been made by Bang, Frosch, and others) it is established that "in all essential points complete agreement exists between these two bacteria."

As we have not had opportunity personally to study cultures from pneumoenteritis in swine occurring in France, we shall not discuss the results of the bacteriological study of this disease published by French investigators, save to remark that different observers have evidently obtained different bacteria from these cases, and that among these bacteria can probably be recognized, although not with absolute certainty from the descriptions, the swine-plague bacillus and the hog-cholera bacillus. We have already criticised Metchnikoff's so-called hog-cholera microbe, which we do not recognize as the genuine hog-cholera bacillus.

It seems to us clear that we have still to await a much more thorough and extensive bacteriological study of the natural disease which occurs in

Europe and is evidently identical with our hog cholera than has yet been furnished us by European investigators with the exception of Bang, before a satisfactory comparison can be made between their results and those obtained in this country on the basis of a much larger experience and more thorough bacteriological work with the natural disease. We trust that such studies will be based upon complete bacteriological examinations of all of the organs of a large number of cases in different epizootics and in different localities, and in the various stages and types of the disease, as has already been done in this country, and that attention will be paid to complicating microorganisms when present.

We have thus far considered the hog-cholera bacillus and its etiological significance. It remains to say something concerning our experience with the swine-plague bacillus about which there has been so much controversy. This bacillus, as we have repeatedly mentioned, belongs to the group of bacteria which has received from Hueppe the name of the bacteria of haemorrhagic septicaemia. It is a totally different species from the hog-cholera bacillus. Its characters were described by one of us (Welch) in 1889, in essential conformity with those described in the publications of the Bureau of Animal Industry. Two main types, with transitions, of virulence can be distinguished; with one kind rabbits are killed by subcutaneous inoculation in from sixteen to thirty hours, usually with enormous multiplication of the bacilli in the blood and organs; with the other kind rabbits die in from two to six days, occasionally longer, with extensive seropurulent infiltration around the seat of inoculation, often with peritonitis, and frequently in these cases with few bacteria in the blood and organs, but an immense number in the inflammatory exudates. Similar variations in virulence occur with other bacteria of this group.

As our observations of the swine-plague bacillus have been either in hogs affected with hog cholera, or in hogs belonging to herds in which hog cholera was prevalent, it became important, as has already been mentioned, to make in every case complete bacteriological examinations, as without such examination one cannot be sure in case only one organism is found that the other has not been overlooked. Such complete examination includes not only the preparation of cover-slip specimens and of plate or roll cultures from all of the organs, but also the inoculation of rabbits or mice with parts of the organs. We can refer to many cases where, if reliance had been placed exclusively upon cultures, or exclusively upon inoculation of animals, we should have failed to detect the presence of the second organism—either the hog-cholera bacillus or the swine-plague bacillus. If both the hog-cholera bacillus and the swine-plague bacillus are present in the part examined, and one of these bacteria greatly predominates in number over the other, cultures are likely to lead to the recognition of only the predominant bacillus, as the second organism may not appear in the culture, or, even if it does, its colonies are distinguished sometimes with such difficulty from those of the other that they can readily escape detection. If a rabbit or mouse be inoculated, the animal will die of swine plague, and the coexistence of hog cholera will thus be unrecognized, if the animal experiment be solely relied upon. In the latter case plate cultures, as has already been mentioned, from the seat of inoculation, may reveal the hog-cholera bacillus associated with the swine-plague bacillus. We have already referred to cases in which the swine-plague bacillus was present in the lungs, or in the lungs and all of the organs, including the intestine, and still the hog-cholera bacillus was found also in the intestine. The separation of virulent hog-cholera bacilli from the intestine in such cases, with general swine-plague infection, is difficult enough in many cases, but much more difficult is the isolation from the intestine of varieties of the hog-cholera bacillus attenuated in virulence. Indeed, so difficult is this that one can rarely feel sure that it may not have escaped recognition. As has already been mentioned, in prolonged cases of hog cholera the hog-cholera bacilli may have disappeared entirely, so far as the most thorough bacteriological examination can determine; and in some of these cases we have found, with the characteristic intestinal lesions of hog cholera, pulmonary or even general infection with the swine-plague bacillus. The intestinal lesions, however, were so characteristic of hog cholera that we were not misled into supposing these cases to be primary swine-plague infections.

Of the epizootics studied by us there were more of hog cholera combined with swine plague than of hog cholera without this complication. It is this frequent association of swine-plague infection with hog cholera that has rendered especially difficult the bacteriological study of hog cholera, and the determination of the pathogenic rôle of each of these species of bacteria. It is this association which makes it of first importance to determine by experimental inoculation of swine, and by the examination of uncomplicated cases of each variety of infection, exactly what the hog-cholera bacillus can do and what the swine-plague bacillus can do, alone or in combination with each other.

We have already stated our conclusions as to the pathogenic and etiological rôle of the hog-cholera bacillus. We have proven by experiments, which we believe were conducted with the strictest precautions to guard against error, that the hog-cholera bacillus can produce all of the lesions of the natural disease which we have enumerated, including characteristic extensive pulmonary hepatization and intestinal buttons, lesions which, so far as we can determine, had not before our experiments been satisfactorily reproduced by inoculation of pure cultures under strict precautions to avoid accidental and previous infection; and we consider that the experimental production of the typical button-like lesions was essential in order to establish positively the causative relation of the hog-cholera bacillus to the natural disease—hog cholera. As the workers in the Bureau of Animal Industry deny that the hog-cholera bacillus can produce pneumonia, save possibly some broncho-pneumonia, our demonstration that this bacillus may cause extensive hepatizations, such as are observed in the natural disease, and which they refer apparently always to the swine-plague bacillus, we believe to be also an important addition to our knowledge of the pathogenic effects of this interesting microorganism.

The class of cases in which we have found most frequently the swine-plague bacillus have been those with pneumonia and fibrinous pleurisy, not very infrequently also fibrinous pericarditis and rarely fibrinous peritonitis. The pneumonia was often very extensive, involving a large part of one or both lungs. The character of the hepatization varied, and was often mixed—gray, reddish gray, red, haemorrhagic, white, necrotic. There was sometimes much interstitial oedema or even solid interstitial exudate in the lungs. Coagulative necrosis with nuclear fragmentation occurs. The swine-plague bacilli were often abundant, either alone, or mixed with hog-cholera bacilli, in the hepatized lung and the exudates on serous membranes. The number, distribution and exclusive presence of swine-plague bacilli in these parts in many cases of the natural disease would lead to the probable inference that they were the cause of these inflammatory lesions; nevertheless, of course, such an inference must be confirmed by experiments on swine before it can be accepted as a positive fact. The swine-plague bacilli were sometimes confined to the lungs or to exudates on serous membranes; sometimes they were found also in larger or smaller number in one or more of the following situations: the blood, spleen, kidneys, liver, lymphatic glands, and intestine; in other words, the infection with these bacteria may be either local or general. We were not able, however, to refer to their presence any definite lesions in these latter situations, save possibly in the intestine, a point which, for manifest reasons, we felt required experimental evidence to decide.

We do not consider it necessary to report here our experiments in inoculating rabbits and other laboratory animals.

Our results from inoculation of swine with pure cultures of the swine-plague bacillus are briefly as follows:

There are marked variations in virulence. Some varieties possessed slight or no virulence when tested on swine. Other varieties were markedly virulent. The duration of life in fatal cases varied from sixteen hours to eight or ten days, rarely more. We have not been in possession of any cultures of this bacillus which were generally fatal to swine by subcutaneous inoculation. We have records, however, of a small number of cases in which fatal general infection exceptionally followed this mode of inoculation, there being extensive inflammatory oedema at the site of inoculation. Intravenous inoculation of very large doses of virulent cultures was generally fatal, of smaller doses was occasionally fatal, often not. By this method of inoculation, even with small doses, we have succeeded in producing multiple serositis (fibrinous pleurisy, pericarditis), both with and without pneumonia. Direct inoculation into serous cavities was sometimes not fatal, although it caused more or less extensive fibrinous inflammation; sometimes it was followed by rapidly fatal general infection with local or multiple serositis. Inoculation directly into the lungs, or better, into the trachea of bouillon cultures furnished us with a number of examples of exquisite characteristic pneumonia, with necrosis, such as has been described, associated with fibrinous pleurisy, sometimes with pericarditis. Like other methods of inoculation, however, this one was not uniformly fatal, even with virulent cultures. Feeding even enormous quantities of swine-plague cultures, as well as the bodies of animals dead of swine plague, produced no effect; and pigs so fed and killed after varying intervals, presented no definite lesions of the stomach or intestine. Inoculation in two cases directly into the intestine after laparotomy was followed by fatal peritonitis without characteristic lesion of the intestine, some of the culture having evidently entered the peritoneal cavity. Combined inoculations of swine-plague and hog-cholera cultures, or inoculations of swine plague before or after inoculation of hog-cholera cultures did not give satisfactory evidence of increased susceptibility of the swine to one or the other of these organisms. Mixed infections were obtained in this way, resembling mixed natural infection.

In fatal cases of pure experimental swine-plague infection of swine we noted in several, but not in the majority of cases, swelling of the follicles, hyperaemia, diffuse redness, and ecchymoses in the intestinal mucosa, particularly of the large intestine, combined often with catarrhal enteritis. Superficial erosions of the haemorrhagic foci and superficial necrosis of these foci were rarely observed. The swine-plague bacillus may produce a haemorrhagic enteritis, as our experiments show. No extensive intestinal diphtheritis, necrosis, ulceration, and nothing resembling the button-like lesions of hog cholera were observed.

In sequestrated sloughs and local inflammatory exudates produced by inoculation of swine-plague bacilli we have found living and virulent swine-plague bacilli as long as two months after the inoculation, the animals having otherwise recovered, and having been killed for the examination. The swine-plague bacilli, therefore, may survive in the animal body longer than in cultures.

The bacteriological examination of pigs dead of experimental swine-plague infection showed the characteristic bacilli pure and in enormous number in the local inflammatory exudates, and in smaller number in other parts, or about entirely from other parts.

We may conclude, therefore, from these experiments that virulent swine-plague bacilli are capable of causing general infection, and more especially local infections characterized by pneumonia and fibrinous inflammations of serous membranes. This conclusion is in harmony with the observations of the distribution of this microorganism and the lesions observed in the natural disease. We find no evidence that the swine-plague bacillus can produce intestinal lesions which are characteristic of hog cholera, although they may cause ecchymoses, hyperaemia, haemorrhagic enteritis, catarrhal inflammation, and probably superficial necroses and erosions. The experimental evidence that they may cause superficial croupous or diphtheritic inflammation of the intestinal mucosa is not conclusive. Notable differences between the hog-cholera bacillus and the swine-plague bacillus are the certainty with which virulent cultures of the former cause intestinal diphtheritis by feeding, and the innocuousness of feeding cultures of the latter to swine, and also the readiness with which pneumonia and fibrinous serositis may be produced by inoculation of the swine-plague bacillus, and the comparative difficulty of producing these lesions with the hog-cholera bacillus.

Theobald Smith considers that natural swine-plague infection is to be regarded as an infectious pneumo-enteritis rather than an infectious pneumonia.[13] The intestinal lesions which he attributes to the action of the swine-plague germ are hyperaemia, haemorrhages, catarrhal inflammation and a peculiar croupous exudation occurring as "circumscribed masses of fibrin easily lifted away from the mucosa, leaving a paler, slightly depressed spot, showing no necrosis of tissue." The last lesion and others which he has described are considered to be different from any produced by the hog-cholera bacillus. So far as we can judge from this description, and particularly from the plate illustrating the lesion, we should not regard this lesion as different from that which may be caused by the hog-cholera bacillus, both experimentally and in the natural disease; and in general we do not consider that Dr. Smith has succeeded in proving or even rendering probable that the swine-plague bacillus produces intestinal lesions of a peculiar character distinguishable from lesions which may occur in hog cholera. In only one instance, published by Theobald Smith, was the experimental inoculation of swine with the swine-plague bacillus followed by an intestinal exudate. This case is described as follows: " In one case, as a result of the peritonitis following an intraabdominal injection, the walls of the small intestine were swollen, inflamed, and a copious, friable yellowish exudate had formed on the deeply inflamed mucosa." This would appear to have been a superficial diphtheritis, and is attributed by Dr. Smith to an extension of the peritonitis.

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[13] Theobald Smith, *op. cit.*, p. 149.

That there may be superficial intestinal areas of necrosis with slight loss of substance on a haemorrhagic basis after experimental inoculation of the swine-plague culture, we have observed and already noted. The well-known relation of epithelial necrosis of mucous membranes to croupous and diphtheritic exudations, and the variety of agencies which may in this way induce intestinal diphtheritis would render it probable that now and then such superficial diphtheritis may be due to the swine-plague bacillus; but at present the evidence for this is in our judgment inconclusive. The fact that feeding swine-plague cultures is not followed by any damage to the intestine is not conclusive evidence that other methods of penetration of the bacilli may not cause intestinal lesions. We have observed the intestinal lesions above specified not only after inoculation of rabbits, but also after intravenous, intrathoracic and intratracheal inoculation of swine with swine-plague cultures, although in most of our experimental cases the intestine was not affected.

The lesions, therefore, which we have succeeded in producing in pigs experimentally by inoculation of the swine-plague bacillus are most frequently pneumonia and inflammations of serous membranes, rarely haemorrhagic enteritis associated with these lesions, and still more rarely by subcutaneous inoculation spreading subcutaneous inflammatory oedema with general septicaemia. We are not able, however, to distinguish an intestinal form of swine plague, either as an experimental or natural disease disassociated from the pectoral form, nor have any cases of natural swine plague been observed in this country analogous to the so-called cutaneous Schweineseuche, although, as already mentioned, we have exceptionally produced this form of the disease experimentally. Of course it cannot be denied that in natural swine plague lesions may occur which do not appear in the experimental disease, as well as lesions in the experimental not observed in the natural affection.

Usually from one and the same pig the swine-plague bacilli isolated in culture are possessed of essentially the same degree of virulence as tested upon animals; indeed, this is in general true of the swine-plague bacilli cultivated from pigs of the same herd affected with the disease. Nevertheless there are exceptions to this, as we have occasionally cultivated from the organs of the same pig, as well as from different pigs of the same herd, extremely virulent swine-plague bacilli, which uniformly kill rabbits by subcutaneous inoculation of small quantities in sixteen to twenty-four hours and bacilli which are fatal to rabbits only after six or eight days. Of course, as already mentioned, it is not necessary to suppose that the degree of virulence possessed by the bacilli, either of swine plague or of hog cholera, isolated at autopsy, is necessarily the same degree which the bacilli primarily possessed at the time of invasion of the animal, as we have instances of modification of virulence of bacteria in the animal body, particularly in long-standing disease. When, for example, we find hog-cholera bacilli greatly attenuated in virulence in pigs dead of chronic hog-cholera, it may well be that the bacilli at the onset of the disease were of the usual virulence, and that the attenuation has taken place in the body of the animal. The same is also applicable to swine-plague infection.

What anatomical differences are there in the natural disease between cases of pure hog cholera, that is, cases in which the hog-cholera bacilli are the only pathogenic organisms present, and cases of hog cholera associated with the presence of the swine-plague bacillus? In our experience, which on this point is in conformity with that of the Bureau of Animal Industry, the cases of combined hog-cholera and swine-plague infections are characterized especially by extensive pneumonia and pleurisy, sometimes by fibrinous inflammation of other serous membranes, in association with the ordinary intestinal lesions of hog cholera. Fibrinous pleurisy and pericarditis may be present in these cases with little or no pneumonia, but in most cases pulmonary hepatization is a marked feature of the combined infection. From the experimental evidence as to the pathogenic properties of the swine-plague bacillus already adduced, as well as from the bacteriological analysis of these cases, it cannot be doubted that the swine-plague bacillus is a cause of such pneumonias and fibrinous serositis.

In many of the cases of pure hog cholera without concurrent swine-plague infection, pneumonia has been absent or there has been only such bronchopneumonia as could be referred to bronchial strongyles which are extremely common in the pigs of Maryland. In a certain number of these cases of pure hog cholera, however, we have found pneumonia of a different type and resembling, in its tendency to become necrotic and its variegated aspect, the pneumonia caused by swine-plague bacilli. Usually the pneumonia was less extensive and less likely to be associated with extensive pleurisy than in cases of combined infection, but occasionally it involved large areas of one or both lungs, and there was fibrinous pleurisy. In these cases we found hog-cholera bacilli alone. It may be urged that in these cases the pneumonia was caused by swine-plague bacilli which had perished. In opposition to this view it may be stated that the swine-plague bacilli may survive at least two months in local exudates in swine as we have shown by experiment, and moreover some of these cases were recent cases, as was shown both by the anatomical and the clinical characters. Again, we have demonstrated experimentally that the hog-cholera bacillus is capable of setting up such pneumonias in swine as those under consideration. For these reasons, therefore, we must differ from the views expressed in the publications of the Bureau of Animal Industry, and admit that the hog-cholera bacilli may cause and do cause pneumonia in swine. We do not wish to be understood as denying that the swine-plague bacilli may die after setting up pneumonia.

In this connection we must again call attention to the cases already mentioned in which we found swine-plague bacilli in hepatized lungs or in the lungs and other internal organs, or in one or more of these situations and in the intestine, and were able to detect hog-cholera bacilli, either virulent or attenuated, only or chiefly in the intestine. We are not aware that cases of this kind have hitherto been described. These were cases of hog cholera with intestinal lesions, most frequently in the form of typical buttons, combined with swine-plague infection. The probable interpretation of these cases is that at an earlier stage of the disease hog-cholera bacilli were present as usual in internal organs, and that in the course of time they disappeared from these organs but remained in the intestine, which is the part generally most intensely affected by this microorganism. That the hog-cholera bacilli may be found in internal organs, even when not demonstrable in the intestines, has also been mentioned.

It is not a wide step from these cases to those in which the hog-cholera bacilli have disappeared entirely from the body, leaving behind characteristic necrotic lesions. In these latter cases also the swine-plague bacilli may be present in the lungs and other organs, including the intestines, or they may be confined to the lungs. We have notes of several cases in which rabbits inoculated with typical necrotic buttons died of swine plague without hog-cholera bacilli demonstrable in any part of the body, including the intestine; but for reasons already mentioned we have no doubt that these buttons were caused by hog-cholera bacilli, and certainly not by swine-plague bacilli. We have also observed several cases with swine-plague bacilli in the lungs, and the rabbits were unaffected by inoculation of the necrotic buttons from the same case, showing that neither virulent swine-plague bacilli nor hog-cholera bacilli were present in these buttons at the time of death. All of these were cases of long standing. It is in these chronic cases especially that we have found hog-cholera bacilli of weakened virulence.

We have evidence, therefore, that hogs which have hog cholera may die infected with swine plague, such infection being characterized generally by pneumonia and pleurisy, when hog-cholera bacilli are demonstrable only in the intestine which presented the lesions of hog cholera; also, that hogs which have had hog cholera may die similarly infected without hog-cholera bacilli being demonstrable at the time of death in the intestine or other organs, although their previous presence is signalized by the presistence of intestinal lesions characteristic of hog cholera. We have also observed combined infection with swine plague and hog cholera in which the hog-cholera bacilli were so atteunated in virulence as not to kill susceptible animals by subcutaneous inoculation. Such attenuated bacilli may be in the intestine, and if confined to this situation the difficulty of demonstrating their presence may be so great that they will readily escape recognition.

These facts, which we are the first to demonstrate by bacteriological examination, must be borne in mind in interpreting cases with intestinal lesions in which only swine-plague bacilli can be detected at autopsy. Laborious as is the examination of the intestine for hog-cholera bacilli, in consequence of the mixture of bacteria there present, such examination, nevertheless, must not be neglected in suspicious cases in which the examination of other parts of the body fails to reveal the presence of these bacilli, and, as our experience has shown, a painstaking bacteriological examination of intestinal lesions will often be rewarded.

We feel compelled to say that not a few of the cases reported in the publications of the Bureau of Animal Industry as cases of pure swine-plague infection, but with intestinal lesions sometimes, to say the least, highly suggestive of hog cholera, were not studied bacteriologically in a manner to make sure of the absence of hog-cholera bacilli from the intestine and not always of their absence even from other parts, and are not interpreted in these publications with sufficient reference to the possibilities of localization of hog-cholera bacilli exclusively in the intestine, or of entire disappearance of demonstrable hog-cholera bacilli from the body, although we would not be understood as saying that these possibilities are wholly disregarded.

This criticism applies only to a certain number of their cases. We have no doubt that some of their cases are examples of swine plague uncomplicated by the coexistence or previous presence of hog cholera in the same animal.

We have no doubt of the occurrence of pure and uncomplicated swine plague as an independent disease, and we have observed several such cases. These cases in our experience were scattered cases in hogs belonging to herds other members of which were affected with hog cholera. The evidence that the cases were pure swine plague lay in the entire absence in some of them of intestinal lesions, in the negative result of the examination of all parts of the body, including the intestine, for hog-cholera bacilli; in the anatomical and clinical evidences that the animals had not previously been affected with hog cholera or other disease; in the presence of the characteristic lesions of swine plague, particularly pneumonia and fibrinous pleurisy, and in the demonstration in pure culture, and in large number, of the swine-plague bacilli in the lesions, and often also in other parts. To assume, under these circumstances, that these pigs were infected or had been infected with hog cholera as well as with swine plague is wholly gratuitous and unsupported by a particle of evidence.

It has not been our fortune to observe an epizootic of pure swine plague. All of our cases, and they have been numerous, have been either combined with hog cholera in the same animal or isolated cases of pure swine plague in herds of swine, some members of which were affected with hog cholera.

A critical study of the reports of the Bureau of Animal Industry on this point shows that their experience has not been widely different from our own, although their interpretation of some of their cases differs from that of similar ones observed by us.

In the various epizootics of swine plague, arranged in eleven groups, studied by Theobald Smith[14] there are unquestionably cases of pure swine-plague infection, but not one of these epizootics can be regarded as free from hog cholera in some of the pigs of the same lot. Thus in the groups numbered II, IV, V, VII, VIII, IX, X, and XI the hog-cholera bacillus was demonstrated in one or more of the pigs, that from IX being attenuated in virulence. In Group I "ulcers or induration of the mucous membrane" of the intestine are stated to be rare, but that they occurred at all makes it probable that some of the pigs had hog cholera. Cultures were made only from the spleen. In only one of the eight cases examined did anything grow, and in this both the colon bacillus and the swine-plague bacillus were found. Group III need not be considered, as only parts of lungs sent to the Bureau were examined bacteriologically. In Group VI the intestinal lesions are stated not to have "differed on the whole from those observed in hog cholera." Cultures were made only from the spleen. The hog-cholera bacillus was not found, unless possibly in a nonvirulent form. In three of the ten cases examined the swine-plague bacillus was demonstrated in the lungs by inoculation of rabbits. The statement as to the intestinal lesions and the incompleteness of the bacteriological examination make it impossible to regard this as an epizootic of pure swine plague.

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[14] *Op. cit.*

We know of no reason why epizootics of swine plague without admixture with hog cholera in at least some of the pigs of the same herd should not occur, but that such epizootics actually do occur in this country remains yet to be demonstrated.

How are we to explain the frequent association of swine-plague infection with hog cholera? The observation made by Theobald Smith, that bacteria closely resembling and probably identical with swine-plague bacteria are frequent inhabitants of the exposed mucous surfaces of healthy swine and other domestic animals, is highly suggestive, and favors the view that the swine-plague bacillus is a secondary invader in many cases of hog cholera, just as in typhoid fever and other primary infections in man we have often enough the secondary invasion of pathogenic bacteria often present on healthy mucous membranes. Such an explanation seems satisfactory for the cases in which hog cholera and swine plague are associated in the same animal, but swine plague may occur in pigs which have no hog cholera. As in the cases of the latter kind thus far observed other pigs in the same herd have hog cholera, one may perhaps suppose either that this hog cholera in other pigs has in some way increased the susceptibility of the exposed pigs, which have not actually contracted the disease, to the swine-plague bacillus, or, more probably, has increased the virulence of the swine-plague bacillus so that it can attack healthy pigs. Assuming some such explanation, simply as an hypothesis without demonstration, it would not be probable that hog cholera is the only agency which could increase this susceptibility or enhance this virulence, but other agencies have not been brought to light in this country.

It remains to consider whether the swine-plague bacillus can be identified with any of the bacteria observed in swine diseases in Europe.

In a recent interesting and important study of swine pest in Denmark, by Bang, the author finds a bacillus which he calls the "vacuole bacillus," and identifies, doubtless correctly, with the swine-plague bacillus of this country. This bacillus was found frequently in swine pest, and is regarded as the cause of the pneumonia and pleurisy associated with swine pest. By inoculating mice and rabbits with bits of organs of pigs dead of swine pest they usually died from infection with this "vacuole bacillus." Pigs inoculated directly into the lungs died of pneumonia without intestinal lesion. Feeding swine with the cultures was harmless. The conclusion reached by this investigation is that in Denmark, as well as in America, swine pest (hog cholera) is often combined with infection with the swine-plague (vacuole) bacillus. This demonstration by Bang of the essential identity of the Danish epizootics with those of this country as regards the two micro-organisms, about which such bitter contests have been waged, is of signal importance, and a confirmation, in important particulars, of the work of the Bureau of Animal Industry and of our own work on this subject.

As already mentioned, both the swine-plague bacillus and the hog-cholera bacillus appear to have been isolated from cases of pneumo-enteritis of swine (hog cholera) in France, but the French investigators have not come apparently to any clear understanding of the relation of these organisms to the natural disease, and often the descriptions of the bacteria concerned are so conflicting or so imperfect that it is difficult or impossible to identify them.

Since the first recognition and description of the swine-plague bacillus in this country, in 1886, in the third annual report of the Bureau of Animal Industry, the important question as to its relation to other bacteria which had been described has been whether or not our swine-plague bacillus is identical with the German Schweine-seuche bacillus. That our hog-cholera bacillus was widely different from the Schweine-seuche bacillus was clear enough even then, and this matter would have continued clear enough if the writings of F. Billings had not confused those whose knowledge of one or both of these organisms was derived only from reading about them.

It was assumed in this first publication of the Bureau of Animal Industry that the swine-plague bacillus is identical with the Schweine-seuche bacillus, which at that time had been shown by Schütz to be the cause of infectious pneumonia in pigs in Germany. The name swine plague (which in the previous report of the Bureau of Animal Industry had been given to hog cholera) was transferred to the disease caused by the new bacillus, on the supposition that it was the cause of epizootic disease in this country, identical with the German Schweine-seuche. Some confusion would have been avoided if another name had been selected for the new bacillus.

We were the first to institute a comparison between cultures of the swine-plague bacillus and the Schweine-seuche bacillus. The latter was obtained from the Hygienic Institute in Berlin in the early part of 1889, and as stated in an article published by one of us (Welch) in 1889 the Schweine-seuche bacillus appeared to be identical with the swine-plague bacillus. This statement was based upon a comparison of the cultures and the inoculation of rabbits and mice. We obtained a second culture of the Schweine-seuche bacillus from the Hygienic Institute, through the kindness of Drs. von Esmarch and R. Pfeiffer, in 1890. This culture was extremely virulent, far more so than the first. It could not be distinguished as regards the morphology of the bacteria, their behavior in culture media, and their pathogenic effects on mice, rabbits and guinea pigs from cultures of our most virulent swine-plague bacillus, but it differed from the swine-plague cultures in being uniformly fatal to pigs by subcutaneous inoculation in moderate doses. In this respect, as in all others, it was like a culture of the Wild-seuche bacillus which we obtained from Berlin. We have never, out of the large number of swine-plague cultures obtained from various epizootics in this country, found one which was usually fatal to pigs by subcutaneous inoculation, although now and then a pig would die from general infection by this method with lesions identical with those after inoculation with the virulent Schweine-seuche germ, namely, spreading local oedema and general septicaemia. The local oedema, however, was not so extensive as in the Schweine-seuche cases. Still a third culture obtained from Kral, in Prague, was again less virulent and indistinguishable from swine plague. Whether, as claimed by F. Billings, under the name Schweine-seuche have been embraced different affections, of which one is identical with Wild-seuche, or, as we think to be more probable, we are to recognize only one Schweine-seuche bacterium with varying degrees of virulence, is not certain. The Wild-seuche bacterium is now considered by some to be identical with that of Schweine-seuche. The Schweine-seuche cultures, both those fresh from the pig and old ones, in the hands of German investigators have also varied decidedly in virulence, as do also those of our swine plague, although no swine-plague culture, even fresh from the pig, has been obtained equalling in virulence, as tested on pigs, the most virulent Schweine-seuche cultures. We observed that pigs which survived repeated inoculation with swine-plague cultures had not lost in any degree their susceptibility to Schweine-seuche. We have had most virulent swine-plague cultures which retained their primary virulence indefinitely in successive cultures.

The only other investigators who have compared, so far as we are aware, cultures of Schweine-seuche and of American swine plague have been Theobald Smith[15] and Afanassieff.[16] Smith used the two Berlin cultures of Schweine-seuche which we gave him. With the first culture he did not succeed in killing rabbits, whereas we produced with it, although not uniformly, the same effects in rabbits as with the swine-plague germ of weakened virulence, that is, after subcutaneous inoculation, local exudate at site of inoculation and peritonitis, the rabbits dying in intervals varying from four to fifteen days. The morphological and biological properties of the two bacteria he found identical. His results with the second culture were identical with our own.

Afanassieff, who worked in Baumgarten's laboratory, obtained his culture of the swine-plague bacillus from F. Billings. It was of weak virulence as compared with many cultures of swine plague which in small doses kill rabbits regularly in less than twenty-four hours. He comes to the conclusion that this culture is identical with that of Schweine-seuche of weak virulence. He made no comparison by inoculation of pigs, which in our experience it is important should be done.

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[15] Smith, *op. cit.*

[16] Afanassieff, *loc. cit.*

Our own conclusion as to the bacteria of Schweine-seuche and of swine plague is that no difference exists between them as regards morphology, cultural behavior, and pathogenic effects on rabbits, mice, and other laboratory animals. Cultures of each occur which are also indistinguishable by inoculation of pigs. The only difference by laboratory experiment which has thus far been brought out is that there occur Schweine-seuche bacteria of higher degree of virulence as tested on pigs than any swine-plague bacteria which have hitherto been isolated from pigs in this country. Another point to be considered in this connection is that Schweine-seuche occurs as an independent disease in Germany without association with hog cholera, whereas swine plague has not been shown to prevail with the same independence as an epizootic in this country. Nor have any cases of cutaneous swine plague analogous to the so-called cutaneous Schweine-seuche been observed in this country as a natural disease.

These points of difference do not suffice to distinguish the bacillus of Schweine-seuche from that of swine plague; at the same time they indicate that we are not at present warranted in asserting unqualifiedly the absolute identity of these bacteria. The whole question as to identity and to separation into varieties and species of various members of the group of bacteria which includes those of swine plague, Schweine-seuche, Wild-seuche, rabbit septicaemia, chicken cholera, etc., is a difficult and perplexing one which has not been satisfactorily settled, notwithstanding the large amount of labor devoted to it. In solving the problem we must take into consideration not only laboratory experiments but also the careful study of the natural diseases caused by the bacteria in question.

REMARKS ON *DIPLOCOCCUS PNEUMONIAE*[1]

In all of the ten cases examined bacteriologically, *Diplococcus pneumoniae* was found and isolated in pure culture. This organism is probably constantly present in croupous pneumonia, and the evidence that it is the specific cause of this disease is very strong. In the cases examined, roll cultures or plate cultures were made from the affected parts of the lungs, from the spleen, from complicating lesions and frequently from the blood and other situations. Nutrient agar, glycerine agar and gelatine agar, according to Guarnieri's formula, were the media usually employed for this purpose. The growth of the pneumococcus on the last named medium is particularly luxuriant. In addition mice and rabbits were inoculated with bits of hepatized lung, with the spleen, etc. In one instance the presence of the pneumococcus would have been overlooked in consequence of the prevalence of other organisms, if the precaution had not been taken to inoculate animals with the tissues. *Diplococcus pneumoniae* cannot be said to be absent simply on the ground of negative results from cultures. This result must be controlled by the inoculation of susceptible animals. Moreover, even the failure to kill mice and rabbits by inoculation cannot be considered conclusive evidence of the absence of *Diplococcus pneumoniae,* for this organism may be present in the human body in cases of croupous pneumonia in a form incapable of killing rabbits or even mice.

One of the most interesting and extraordinary properties of this organism is the variation in its virulence when tested upon animals. All who have experimented with cultures of *Diplococcus pneumoniae* have called attention to its rapid loss of virulence when cultivated in artificial media. It is important to note that a progressive diminution of virulence may occur likewise in the lungs and other organs where the pneumococcus is present in cases of croupous pneumonia. One case of croupous pneumonia dying in the stage of gray hepatization showed very numerous colonies of *Diplococcus pneumoniae,* mixed with a few colonies of *Staphylococcus aureus* in agar plate cultures from the consolidated lung. A rabbit inoculated with a piece of the lung, died on the fifth day with the extensive local reaction and large spleen. The pneumococci were abundant near the seat of inoculation, and were found in considerable number, although fewer than usual, in the blood. Plate cultures (agar) made from the swollen spleen (after burning thoroughly the surface) of this patient showed in very large number, colonies of the pneumococcus. No other species of organism was present in these cultures from the spleen. The same pneumococcus was likewise observed in cover-glass preparations from the fresh spleen. The organism isolated from the spleen conformed in every particular as regards its morphology, its behavior in artificial culture media, its short vitality, its color reactions to the pneumococcus found in the lung, the so-called Fraenkel-Weichselbaum pneumococcus. Nevertheless it was devoid of its customary virulence, for a mouse and a rabbit inoculated subcutaneously with a bit of the spleen survived, and mice and rabbits inoculated with pure cultures of the organism likewise survived, the only reaction being a small abscess or purulent infiltration of the tissues at the point of inoculation. In this case, therefore, *Diplococcus pneumoniae* was present in large numbers in the hepatized lung in a weakened state as regards its virulence and in the spleen in a condition incapable of killing rabbits and even mice. Every grade of virulence was observed in the pneumococci found in the different cases, from the degree just described as incapable of killing mice and rabbits up to the degree in which rabbits were killed in 36 to 48 hours by an acute septicaemia. In two cases the pneumococcus did not kill rabbits but it did kill mice, so that the latter animals are more susceptible and are to be preferred for inoculating tissues in cases of croupous pneumonia. In three cases rabbits survived the inoculation for more than five days, the longest duration being twelve days. Inoculation of rabbits with exudation present in the bronchi or in the trachea sometimes caused a rapidly fatal sputum-septicaemia even when the pneumococcus present in the hepatized lung showed weakened virulence. In general the impression was obtained that the most virulent forms of pneumococci are to be found in the sputum, in the freshly hepatized lung or at the margin of an advancing pneumonia, whereas the pneumococci present in the advanced stages of hepatization and in the spleen are likely to be less virulent. This progressive diminution in virulence as tested upon animals, of the pneumococcus in the consolidated lung and other organs in cases of croupous pneumonia is surely a significant circumstance. In no instance was the pneumococcus isolated in a state capable of producing pneumonia in dogs, although not less than 5 c. c. of a bouillon culture 24 hours old (in the thermostat at 37° C.) of the pneumococcus, obtained from a rabbit dead 40 hours after inoculation, had been injected directly into the lung. Other observers, however, have obtained positive results by this method of inoculation. Others have found pneumococci producing more rapidly fatal septicaemia in rabbits than resulted from any of the cultures obtained from the cases examined.

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[1] Report of remarks [abstract] before the Johns Hopkins Hospital Medical Society, Baltimore, February 17, 1890.

Johns Hopkins Hosp. Bull., Balt., 1889-90, I, 73-74.

In six of the ten cases *Diplococcus pneumoniae* was found in pure culture in the hepatized lung as determined by microscopical examination and by plate or roll agar cultures; in two cases it was present in combination with *Staphylococcus pyogenes aureus;* in one case, in addition to the pus staphylococci, *Streptococcus pyogenes* was present in considerable number; and in one case with gangrenous foci there were present, besides *Diplococcus pneumoniae* and the pus staphylococci, a short oval bacillus and a long slender bacillus not developing in plate cultures (aerobic cultures). In one of the cases in which *Staphylococcus pyogenes aureus* was present, several small abscesses had formed in the hepatized lung. In four cases *Diplococcus pneumoniae* was found in pure culture in the spleen, which in all of these instances was swollen and soft; in one case it was isolated from the kidney and in one from the heart's blood. In all instances (five) in which the pleuritic exudate accompanying acute croupous pneumonia was examined by culture methods *Diplococcus pneumoniae* was found in this exudate. In three cases of empyaemia following acute croupous pneumonia (these do not belong to the ten cases now under consideration) *Diplococcus pneumoniae* was found; in two in pure culture, in one in combination with *Staphylococcus pyogenes aureus* and albus. The pneumobacillus of Friedländer was not found in any case.

A convenient way of staining the capsules is to treat the cover-glass specimens, dried and treated in the usual way, with glacial acetic acid and then without washing off the acid to drop on the cover-glass aniline-oil gentian-violet, which can be allowed to drain off and can be renewed two or three times. Attention should be called to the broad pale rods corresponding to empty capsules sometimes found in the fluid of empyaemia following pneumonia. These may be 10-30 mm. long and 2-3 mm. broad. They stain faintly, may be entirely empty or may contain one or more deeply stained pneumococci. They correspond to the capsular substance, around rows of pneumococci, the cocci having partly or wholly disappeared.

For staining sections, Weigert's fibrin stain has given most excellent results. A method which yielded good and quick results was to dry thin frozen sections from the fresh organs upon the slide and then to heat them for half an hour at a temperature of 110° to 115° C. These can then be stained like cover-glass specimens. This method is often employed in the laboratory for staining fresh tissues in general for microorganisms.

The statements of Fraenkel and others were confirmed as to the behavior of *Diplococcus pneumoniae* in artificial culture media, its susceptibility to slight changes in the reaction and composition of the medium, its brief vitality, etc.

The frequent presence of the pneumococcus in the saliva of healthy persons is upon the whole an assistance to us in the explanation of the various factors concerned in the causation of croupous pneumonia.

THE ETIOLOGY OF ACUTE LOBAR PNEUMONIA, CONSIDERED FROM A BACTERIOLOGICAL POINT OF VIEW[1]

Members of the Faculty.—I had the honor at the last annual meeting of this Faculty of addressing you upon the "Causation of Diphtheria," and I endeavored then to present the results of the bacteriological study of this disease in a manner which might be helpful to the practitioner of medicine. It has seemed to me that it may be acceptable on this occasion to consider "Acute Lobar Pneumonia" from a similar point of view.

Various names have been given to the bacterium which we now believe to be the specific cause of acute lobar pneumonia, such as *Micrococcus pasteuri* (Sternberg), micrococcus of sputum septicaemia (A. Fraenkel), diplococcus or diplobacillus or pneumococcus of Fraenkel and Weichselbaum, *Diplococcus pneumoniae* (Weichselbaum), *Micrococcus* or *Diplococcus lanceolatus capsulatus* (Foà and Bordoni-Uffreduzzi), *Micrococcus pneumoniae cruposae* (Sternberg). Of these names *Micrococcus* or *Diplococcus lanceolatus, Diplococcus pneumoniae* and pneumonia coccus or pneumococcus have gained the widest currency and will be used in this article. Perhaps the name *Micrococcus lanceolatus* is the least objectionable. Such names as *Pneumococcus, Micrococcus pneumoniae cruposae* suggest an exclusive relationship of this organism to croupous pneumonia, whereas this same organism is concerned in the causation of epidemic cerebrospinal meningitis and many other affections independent of pneumonia.

Micrococcus lanceolatus was discovered by Sternberg in September, 1880, by inoculation of rabbits with his own saliva. It was next found by Pasteur in December, 1880, by inoculating rabbits with the saliva of a child dead of hydrophobia. Pasteur's observations were the first to be made public in January, 1881. Sternberg's first publication on this subject appeared in April, 1881.

No little sensation was made by Friedländer's publication in November, 1883, in which he described cultures of a bacterium to which he gave the name pneumococcus. We now know that the so-called pneumococcus of

At this time there was no suspicion that this micrococcus of the saliva is concerned in the causation of lobar pneumonia.

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[1] President's address before the Medical and Chirurgical Faculty, Baltimore, April 26, 1892.

Tr. M. & Chir. Fac. Maryland, Balt., 1892, 1-29.

Friedländer is an organism entirely distinct from *Micrococcus lanceolatus* and is to be regarded as a bacillus. This pneumobacillus of Friedländer is probably in no way concerned in the causation of genuine acute lobar pneumonia in man. Friedländer's method of cultivation in nutrient gelatine at ordinary temperatures precluded his obtaining the genuine pneumonia coccus. The historical importance of this publication of Friedländer, is that he was the first to describe cultures obtained from croupous pneumonia by using Koch's solid nutritive media, that his pneumobacillus was widely accepted until the year 1886 as the specific cause of croupous pneumonia and especially that his results gave an impetus to the further bacteriological study of this disease by modern methods.

For a while there was much confusion of the genuine pneumonia coccus with Friedländer's bacillus. Thus the observations of Talamon in 1883, of Salvioli in 1884 and of Sternberg in 1885, unquestionably pertained to *Micrococcus lanceolatus* but these writers thought they were working with the pneumococcus which had been described by Friedländer.

This period of confusion was brought to an end by the exhaustive and fundamental researches of A. Fraenkel, published in 1886. His articles furnished a full and accurate description of the leading characters of *Micrococcus lanceolatus,* rendered probable its causal relation to acute lobar pneumonia, separated this organism clearly from the pneumobacillus of Friedländer and recognized its identity with the micrococcus of sputum septicaemia.

Not less important were the independent investigations of Weichselbaum, published in October, 1886, and based upon the study of a much larger number of cases. Foà and Bordoni-Uffreduzzi also deserve mention among the pioneer investigators of this period. In the same year they reported their observation of lanceolate diplococci in the exudate of epidemic cerebrospinal meningitis. They identified correctly this microorganism, which they called meningococcus, with Fraenkel's pneumonia coccus.

Since these fundamental researches, each year has been rich in contributions to the literature of our subject.

As the name lanceolate coccus implies, the typical form of the pneumonia coccus is oval, with one end somewhat more tapering than the other. It is often compared in shape to the flame of a candle or to a grain of barley. Regularly oval and spherical forms, however, are not uncommon and there may be actual rods or bacilli. The propriety of calling this organism a coccus is open to question. Its typical form is transitional between a coccus and a bacillus. Usage is in favor of calling it a coccus, but some good authorities prefer to regard it as a bacillus.

The arrangement in pairs is so characteristic that this has given origin to the name *Diplococcus pneumoniae.* Pneumococci not infrequently appear in short chains and, especially in old cultures and when devoid of virulence, they may grow in long and curved chains, regular streptococci.

In fact, there is good reason to believe that *Micrococcus lanceolatus* belongs to a species presenting varieties which are represented by every transition from single and double cocci to long streptococci and which are endowed with varying degrees of virulence and that some of these varieties are almost constantly present in the mouth. This relation of the pneumococcus, which ordinarily in croupous pneumonia appears as a diplococcus, to streptococci is of much interest in view of the increasing importance attached to primary and especially secondary streptococcus infections. I shall have occasion throughout this article repeatedly to refer to the extraordinarily variable characters of the pneumonia coccus, which render a concise and accurate description of the organism difficult.

The third morphological characteristic of the pneumonia coccus is the presence around it of a clear gelatinous capsule which can be stained with various aniline dyes. The combination of the three traits mentioned suggested the descriptive name *Diplococcus lanceolatus capsulatus.* Capsules are as a rule readily demonstrable only around the cocci growing in the animal body, although they may be present also in cultures.

Degenerating and dead pneumococci, which are very common in old inflammatory exudates caused by this organism, often leave behind them empty or faintly staining capsules.

Micrococcus lanceolatus is not motile. It never forms spores. It stains well with Gram's and Weigert's fibrin stain.

It grows best at temperatures approaching that of the human body. Below 24° C. (75° F.) the growth may cease, but sometimes there is development at as low a temperature as 18° C. (64.4° F.), especially when the organism has been cultivated for some time outside of the body and has lost virulence. Nutrient gelatine, therefore, which melts at 22°-24° C., is not a medium adapted for obtaining first cultures of the pneumococcus, and as already mentioned it was doubtless in consequence of the use of this medium that Friedländer failed to isolate the genuine micrococcus of croupous pneumonia.

The pneumococcus is capable of growing on all of our ordinary culture media but there is no other known organism which will grow on so many media and at the same time is so particular as to the exact composition of the medium. Slight differences in the reaction of the medium, in the quality of the peptone or of the meat used to prepare the medium often determine whether or not growth takes place.

The most suitable and generally employed culture medium for this organism is feebly alkaline nutrient agar or a mixture of agar and gelatine. The growth is usually delicate and grayish in color, but it may be more abundant and opaque. Gelatine is not liquefied. Milk is usually soured and coagulated. No visible growth on potato usually appears. The culture in bouillon may be diffusely cloudy or in the form of a granular sediment. Growth occurs without as well as with the presence of free oxygen.

The variability in the properties of this coccus is manifested not less in its behavior in culture media than in other respects. Decided modifications in cultural characters, particularly as to luxuriance of growth and capacity of development at low temperatures can often be brought about by artificial cultivation. Abundant growth at low temperatures is generally associated with loss of virulence and is often observed in late generations of artificial cultures of primarily virulent pneumococci.

One of the most striking properties of the lanceolate coccus both in the animal body and especially in cultures is its short viability, a property, however, which like most of the others is not without exceptions. The maximum development in cultures at body temperature is attained in twenty-four hours or less. After this there is usually progressive and rapid death of the bacteria so that at the end of four to seven days the cultures are usually dead. Cultures capable of surviving two weeks or more are generally not virulent, but to this rule there are exceptions.

In order to be sure of keeping the cultures alive they should be planted over every day or two. But even this care will not as a rule preserve the virulence of the organism.

After the third or fourth generation it is not uncommon to find marked diminution of virulence and after ten generations, and often sooner, virulence has generally nearly or entirely disappeared. To keep up the virulence of the organism the plan is usually adopted of transmitting it frequently through rabbits, but even this will not always succeed.

This short viability and rapid loss of virulence give to *Micrococcus lanceolatus* a quite exceptional position among pathogenic bacteria and they constitute an annoying obstacle to systematic experiments with this organism.

The limited duration of life of the lanceolate coccus is a matter of great practical interest. It is capable of longer life in the virulent state in susceptible animals and in human beings than in artificial cultures but it is apt to die even in the living body sooner than most other pathogenic bacteria. Dead pneumococci are found often in large number in empyaemas and in the exudate of croupous pneumonia. This short vitality is doubtless one reason why empyaemas and other local inflammations caused by the lanceolate coccus usually afford a more favorable prognosis than those caused by the longer lived *Streptococcus pyogenes* and why a single aspiration of a pneumococcus empyaema may be followed by a permanent cure. But here as well as with other properties of this peculiar microorganism it is necessary to keep in mind the frequent exceptions to the rule, instances occurring in which virulent pneumococci are found in empyaemas and other inflammatory exudates at least six months old.

The pneumococcus may suffer a progressive loss of virulence during the course of croupous pneumonia so that at the end of the disease the cocci may be nearly devoid of virulence, but this behavior is not sufficiently constant to establish a rule.

Pneumonia cocci in blood and sputum are in general tolerably resistant to drying, living and retaining their virulence in the dried state sometimes for at least four months, although they may die much sooner. This resistance to desiccation as well as other reasons indicate the propriety of destroying or disinfecting pneumonic sputa.

According to Sternberg the pneumonia coccus is killed by exposure for ten minutes to a temperature of 52° C. (125.6° F.). The action of sunlight is injurious to this as well as to other bacteria.

Experiments upon animals and observations on human beings prove that *Micrococcus lanceolatus* is an organism of the most manifold and varied pathogenic possibilities. It is the cause in human beings of a large number of affections formerly regarded as etiologically distinct. Before considering these it is desirable to say something concerning the results obtained by inoculation of animals.

We meet under natural conditions and we can readily obtain by artificial cultivation pneumococci totally devoid of any virulence to animals and at the other extreme we find pneumococci capable of killing rabbits by septicaemia in eighteen hours or less. Between these extremes occur pneumococci of all possible degrees of virulence and capable of causing manifold pathological lesions.

The experimental results vary with the animal, with the number and virulence of the bacteria inoculated and with the site of the inoculation. Rabbits and mice are the most susceptible animals. The principal symptoms which may be observed in rabbits inoculated with virulent pneumococci are fever, diarrhoea, albuminuria and shortly before death convulsive seizures. Pulmonary oedema sometimes accompanies the death agony.

Septicaemia with multiplication of the cocci in the blood is regarded as the leading type of experimental pneumococcus infection of rabbits. With this the rabbits usually dies in one to three or four days. The more common and important lesions are serous, fibrinous or fibrinopurulent exudation around the point of inoculation if this has been subcutaneous, swelling of the spleen, which may be either hard or soft, small, opaque foci of necrosis in the liver, fatty degeneration of the heart, ecchymoses, and sometimes hyaline thrombi in the renal capillaries. Sometimes there is fibrinous inflammation of the peritoneum or other serous membrane. Cocci are usually abundant in the blood but they may be scanty. They are always numerous in recent local exudates.

The attempt has been made to establish distinct biological varieties of *Micrococcus lanceolatus* on the basis of diverse pathological effects in these septicaemias, especially on the basis of the consistence of the spleen and the character of the exudate at the point of inoculation. But these alleged distinctive characters have not been found to be sufficiently constant to justify a sharp separation into distinct varieties.

A second type of pneumococcus infection in rabbits, not sharply separated from the septicaemic type, is that characterized by spreading, localized, usually multiple fibrino-purulent inflammation without many cocci in the blood. There is evidence that this exudative type depends upon weakened virulence of the cocci. Rabbits affected with this form of the disease live usually four to fifteen days and sometimes longer. Very virulent pneumococci may produce this exudative type in rabbits rendered partly immune from infection. In the majority of cases there are associated with extensive subcutaneous exudation spreading from the site of inoculation, similar fibrinopurulent inflammation of one or more serous membranes and sometimes hepatized pulmonary areas. The spleen is often little or not at all changed. Cocci are usually few in the blood but in enormous numbers in the exudates, in which in the course of time many degenerate and die. The animal becomes emaciated and anaemic, commonly with marked leucocytosis.

Croupous pneumonia in human beings belongs to the exudative rather than to the septicaemic type of pneumococcus infection. Inasmuch as the occurrence of such spreading inflammations with little invasion of the blood by pneumococci is experimentally shown to depend chiefly upon the relation existing between the virulence of the coccus and the resistance of the animal and as we cannot suppose that the pneumococci which cause croupous pneumonia are of weakened virulence, the inference seems warranted that man ranks among those relatively insusceptible to this organism.

A third type of disease caused by subcutaneous inoculation of *Diplococcus pneumoniae* is the formation of an abscess at the point of inoculation. This occurrence indicates either still less virulence than in the preceding types or, with the same virulence, greater resistance on the part of the animal. Rabbits which have been rendered sufficiently immune to be protected from septicaemia often acquire a local abscess at the point of inoculation. Under these conditions *Micrococcus lanceolatus* is an exquisitely pyogenic microorganism, as it may also be in man. Usually the animal recovers after evacuation of the abscess.

A rare form of experimental pneumococcus disease is pyaemia, with multiple suppurative foci in the joints and other parts of the body.

It is noteworthy that in animals as well as in man *Micrococcus lanceolatus* is capable of producing not only fibrinous but also genuinely purulent inflammations of the serous membranes.

The efforts to reproduce in animals a form of pneumonia in all respects identical with acute lobar pneumonia as it occurs in man have been only moderately successful. To accomplish this it is evident that the animal must not die of quick septicaemia. Hence these experiments have been made either with virulent cultures on relatively insusceptible animals such as the dog, or with attenuated cultures on rabbits. A few experimenters claim to have produced by intrapulmonary or tracheal inoculations typical lobar fibrinous pneumonias, but the usual result if the organism is sufficiently virulent, is the production of pleurisy and pericarditis with or without circumscribed areas of pulmonary hepatization.

So far as known the domestic animals are not subject to a form of pneumonia etiologically and anatomically identical with croupous pneumonia of man. We do not usually in our experimental tests with pathogenic microorganisms on animals reproduce the exact counterparts of human diseases nor is it necessary or to be expected that we should do so in order to rest satisfied with our experimental evidence. We possess conclusive experimental proof that the pneumococcus is capable of producing spreading inflammatory exudates with all of the histological and bacteriological characters of the exudate in croupous pneumonia as it occurs in man.

As *Micrococcus lanceolatus* is the cause of many cases of cerebrospinal meningitis (hence the synonym, meningococcus), the attempt has been made by submeningeal inoculation to reproduce this lesion in animals and with occasional success.

Endocarditis which is a very rare result of simple subcutaneous or intravenous inoculation of the pneumococcus can be readily produced by the well known method adapted to cause infectious endocarditis, viz.: by first injuring the valves by means of a sterilized probe passed from the carotid artery into the left ventricle and then injecting the cultures. This is an illustration of the predisposing influence to localized infection of *locus minoris resistentiae.*

Various other special localizations such as arthritis and periarthritis, ostitis and otitis media have been produced by special methods of inoculation, in order to demonstrate the possibility of reproducing in animals similar affections caused by the same organism in human beings.

It is evident from the foregoing review, which is only partial, of the pathogenic effects which may be produced by *Micrococcus lanceolatus* in animals that we possess a rich mass of experimental data to aid us in explaining the multiform manifestations of infection by this microorganism in human beings. The pathogenic effects produced are a function on the one hand of the number and virulence of the infecting cocci and on the other hand of the degree of susceptibility of the individual and according to these differences the result is now septicaemia, now single or multiple serofibrinous or fibrinous or fibrinopurulent or purulent or haemorrhagic exudations or now a localized abscess.

The transmission of pneumococci from mother to foetus has been demonstrated both in human beings and in animals. It is interesting to note that in the few hitherto reported observations in human beings in which the pneumococci have been demonstrated in the embryos of mothers affected with croupous pneumonia, there has been no pneumonia in the foetus although the organisms were present in the blood, whereas in infants congenitally infected who had lived some hours after birth, actual pneumonia has existed. Netter concludes thas it is necessary that the child should have breathed in order to acquire localization of pneumococcus infection in the lung. This is an illustration, and there are others in the case of different diseases, of the difference in susceptibility between the embryo and the infant after birth.

To produce intra-uterine infection of the foetus the pneumococci must of course have circulated in the blood of the mother and have broken through the placental barrier, which-as is well known is a perfect physiological filter for inanimate particles. But living microorganisms are capable of damaging in various ways the placental tissue and opening a passage into the foetal vessels. Some microorganisms do this often, others only exceptionally or not at all.

It is probable that the transmission of pneumococci from mother to foetus is exceptional in the case of human beings. The toxic substances produced by microorganisms, being soluble, can of course pass much more readily through the placental vessels to the foetus than the microorganisms themselves, and these poisons often cause the death of the foetus. It is well known that pneumonia developing during pregnancy is likely to bring about miscarriage. The same is to be noticed in mice and rabbits inoculated with virulent pneumococci. Neither in human beings nor in animals does abortion necessarily attend pneumococcus infections.

Pneumococci have been found in the milk of rabbits inoculated with the virulent organism and suckling rabbits have been infected by the ingestion of this milk. Pneumococci have been detected also in the milk of women affected with croupous pneumonia. As to the frequency of this occurrence we have not sufficient information.

Evidence is constantly accumulating that pathogenic bacteria do injury chiefly by their toxic chemical products. It was thought at one time that the most important of these poisonous products are crystallizable alkaloidal substances called ptomaines, but it is now known that the toxic products which produce the more characteristic and specific manifestations of an infectious disease are of an entirely different nature. These more specific poisons are believed for the most part to be proteids and are called toxalbumins or toxic proteids. We know very little of their chemical constitution and identify them chiefly by their biological effects. They have not been separated in a state of purity but are contained usually in albuminous precipitates. They are formed both in the body of the infected animal and in cultures, often more abundantly in the former. In fact with some pathogenic bacteria which give evidence of the formation of toxins in the infected body, there has been difficulty in demonstrating poisonous substances in cultures.

The most powerful toxic bacterial proteids are produced by the bacilli of diphtheria and of tetanus which multiply as a rule only or chiefly locally near the point of entrance. In the experimental septicaemic infections, such as may be caused by the pneumococcus, where there are abundant invasion and multiplication of the bacteria in the circulating blood, we do not find such concentrated and powerful poisons as in the more distinctively toxic diseases like tetanus and diphtheria.

Cultures of the pneumonia coccus as a rule do not contain in a concentrated form toxic substances. As much as thirty to forty cubic centimeters of beef broth cultures of virulent pneumococci, sterilized or deprived by filtration of bacteria, can sometimes be injected into the circulation of rabbits without grave symptoms. Cultures concentrated by evaporation at low temperature cause death when injected in smaller quantities. As might be expected with an organism so variable in all of its properties, some cultures are more poisonous than others, so that much smaller quantities than those mentioned may cause intoxication.

There is clinical evidence both for human beings and animals that the pneumococcus may produce toxic substances in the living body. In fact we find in the blood of rabbits dead of pneumococcus infection, more concentrated toxic substances than we are able to demonstrate in cultures. The blood of human beings affected with croupous pneumonia is often highly poisonous for rabbits, three to six cubic centimeters of such blood injected into the circulation of rabbits sufficing to cause grave symptoms and even speedy death. This blood may retain its toxic properties for a time after the crisis.

With the exception of the unconfirmed observation of Bonardi no toxic ptomaines have been found in pneumococcus cultures. There have, however, been obtained from cultures and from the blood and tissue juices of animals dead of infection albuminous substances, possessing poisonous properties. It is believed therefore that the specific poison produced by the pneumococcus, as well as the poisons of most pathogenic bacteria, belong to the group of toxic albumins. It has received from the Klemperers the name pneumotoxin. The name is a convenient one but it must not be inferred that it refers to any substance which has hitherto been isolated in a state of chemical purity or which has been demonstrated to be of an albuminous nature.

Grave constitutional symptoms and some of the local lesions of pneumococcus infection are referable to the poisonous substance or substances called pneumotoxin. It is questionable, however, whether this substance is concerned in the production of local inflammatory exudates. These exudates must be caused by bacterial products possessed of powerful positive chemotactic properties, that is, of the power of attracting leucocytes. experiments indicate that the specific toxalbumins repel rather than attract leucocytes. It is held by Buchner and others that substances called bacterioproteins, set free from degenerating and dead bacteria, are the chief agents which manifest positive chemotactic properties. This view fits in with the fact that it is especially bacteria of weakened virulence in susceptible individuals or virulent bacteria in more resistant individuals which attract leucocytes and set up local inflammations. There is evidence however, that living and vigorous bacteria may also attract leucocytes so that we cannot accept Buchner's observations as excluding other explanations of the accumulation of inflammatory products, more particularly leucocytes, around bacteria.

Whether we suppose that the positively chemotactic substances are derived from pneumococci already damaged by the living cells and fluids of the body in accordance with Buchner's view, or from still active and thriving pneumococci, there is good reason to believe that these substances are not identical with the toxalbumins which, by their absorption into the circulation, cause the graver constitutional symptoms often observed in croupous pneumonia and other pneumococcus infections. The variability in these clinical manifestations may depend partly on the susceptibility of the patient and partly on the virulence of the pneumococcus. Varying degrees of virulence of a microorganism are to be interpreted usually as varying capacity to produce toxic substances.

A class of bacterial products of great importance are those which are capable of producing immunity. Rabbits can readily be rendered insusceptible to fatal doses of virulent pneumococci. All of the known principles of producing artificial immunity have been successfully employed in protecting rabbits from *Micrococcus lanceolatus.* These principles of immunization are:

1. Inoculation of small, not fatal, quantities of virulent cultures of the specific microorganism causing the disease.

2. Inoculation of the specific microorganisms partly or wholly attenuated in virulence. The ways by which virulence can be weakened or annulled are various, and consist chiefly in exposure to agencies injurious to bacteria. In the case of the pneumococcus we often meet under natural conditions the organism so weakened in virulence as to serve as a vaccine against the virulent variety.

3. Injection of products of the bacteria causing the disease, either products contained in sterilized cultures or in the germ-free filtrate of cultures or products obtained from the body of an infected individual. These substances may be used when still toxic, or better, as a rule, after diminution or removal of their toxicity by heat, mixture with chemical antiseptics or other means. This class constitutes the so-called chemical vaccines in distinction from the living vaccines just described.

4. Injection of the blood serum or other fluids from animals artificially rendered immune from the disease. This method is different in principle from the first three, and the resulting immunity presents important peculiarities. As this immune serum possesses also curative properties, it is called curative or healing serum.

Inasmuch as it is by their chemical products that bacteria confer immunity, as well as cause disease, the distinction which appears in the preceding classification between living vaccines and chemical vaccines is not so fundamental as at first glance it might appear.

The immunizing bacterial products are believed to be proteids, and especially of the class derived from the bacterial cells, but we recognize them by their physiological properties rather than by any known chemical tests. The relation in which they stand to the toxic proteids is not definitely known. Inasmuch as the vaccinating power of sterilized or filtered cultures deprived partly of toxicity by heat or by other means is greater than that of the unaltered toxic products, it is held that the immunity-conferring products are either distinct from the toxic products or are derived from them.

When we introduce vaccinating substances, belonging to the first three methods just described, immunity does not ensue at once, but there is a period of reaction, often severe, with fever, local inflammation and other symptoms, which precedes the establishment of immunity. In the case of the pneumococcus this period of reaction lasts three or four days after intravenous injection and about two or three weeks after subcutaneous injection. The resulting immunity may be augmented by repeated successive injections of vaccines of increasing strength and finally of increasing quantities of the virulent organism. In this way rabbits may be protected against subsequent inoculation with several times the fatal dose of virulent pneumococci. Such very high degrees of immunity as have been secured from tetanus by vaccination, have not, however, been obtained from pneumococcus infection. The immunity may last at least two years, but usually disappears sooner.

The protection which is afforded by injecting the blood serum of an animal already rendered by vaccination or by recovery from the disease immune from the pneumococcus rests upon a different principle from that afforded by vaccination with the living microorganism or its products. By the former method we inject a fluid which is already endowed with properties upon which immunity depends, whereas by the latter methods these properties must be developed before immunity is established. In using immune serum we simply transfer a part of the immunity possessed by one animal to another, or at least we introduce substances which quickly bring about that change in the body upon which immunity depends. Hence this is called passive immunity, in distinction from the active immunity induced by vaccination. Passive immunity appears at once or within a few hours after the injection of immune serum, and is not preceded or attended by any noteworthy reaction. To compensate for this speedy and safe development, passive immunity last a shorter time than active immunity, and is in direct proportion to the quantity of immune serum introduced and the degree of immunity possessed by the animal from which the serum is derived. It is only a fraction of the immunity of the animal yielding the serum.

Acquired immunity is the results of a specific reaction of the living body, which in the case of the pneumococcus can be produced only by the direct or indirect products of this organism.[2] Hence when we find that the blood and fluids of persons recently convalescent from pneumonia is capable of protecting rabbits from infection with the pneumococcus, and that this property is absent from the blood before an attack of pneumonia, we feel justified in inferring that the pneumococcus has been operative in the causation of the pneumonia.

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[2] To this general statement an exception must be admitted if Bonome's assertion be confirmed that rabbits may be protected from the pneumococcus by the sterile filtrate of cultures of the bacterium of rabbit septicaemia.

Different explanations of artificial immunity from pneumococcus infection have been offered. One theory is that the blood and fluids of the immune individual have acquired the power of destroying the specific poison or pneumotoxin. A second theory is that the blood and fluids exert a direct germicidal action on the pneumococci. A third theory is that the leucocytes and other cells act as phagocytes and eat up the bacteria.

The first or antitoxic theory is advocated especially by the Klemperers. According to this view immunity from pneumococcus infection depends upon the same principle as has been proven for immunity from tetanus and diphtheria. The substance in the blood and fluids which acts as a direct antidote to the specific bacterial poison is called antitoxin, in the present case antipneumotoxin. The Klemperers explain the crisis of pneumonia by the sudden or critical production of the antitoxin. As soon as the poisonous weapons of bacteria are destroyed, these organisms, even if they survive for a time, are reduced to the level of ordinary saprophytes, and are as incapable of doing harm as a venomous snake is after extraction of its poison fangs.

The observations upon which the Klemperers base their fascinating theory, are opposed by those of several other investigators, and we cannot by any means consider the antitoxic theory of immunity from pneumococcus infection as proven.

Nor are the results of different experimenters regarding the second and third theories in accord. As might be predicted, Metchnikoff and his students find abundant evidence for the phagocytic theory of acquired immunity from the pneumococcus as from all other infections. As with immunity from many other infectious diseases, it cannot be said that we possess at present any thoroughly demonstrated explanation of the basis of immunity from this organism.

A most important characteristic of the blood serum and some other fluids from animals or human beings who have recently acquired immunity from pneumococcus infection is that they are capable not only of protecting a susceptible animal from subsequent inoculation with the organism, but also of protecting after such inoculation. This is the so-called healing property which, in some degree, belongs to the blood and fluids in most cases of solid acquired immunity from infectious bacteria. Protection after reception from the virus, however, is by no means so simple a matter as mere immunization before reception.

When the immune serum is introduced soon after the inoculation with the virulent organism, it must be given in larger quantity than is required a few hours before inoculation. With each succeeding hour the difficulty of conferring protection increases, and as soon as the symptoms have appeared only large doses of serum of high immunizing power offer chance of recovery. In rapidly fatal pneumococcus infections, soon after the onset of the symptoms it is no longer possible to rescue the animal. With slower infections cure has been effected when treatment has begun twenty-four hours after the appearance of the first symptoms.

It is well to have clearly in mind the factors which, according to our present knowledge, control the results of this so-called blood serum therapy. These factors relate to the dose, the immunizing power of the serum used, and the stage and intensity of the disease when treatment is begun. The larger the animal the greater must be the dose, and in the case of tetanus, where the principles of serum therapy have been worked out more fully than for any other disease, the dose is in approximately direct ratio to the weight of the individual. With serum, therefore, of only moderate immunizing power, the quantity required to protect a large animal or man may be so great that its introduction would amount to a transfusion of blood, or might exceed any amount which could be injected. It is of the first importance, therefore, to secure the highest possible degree of immunity in the animal yielding the serum, or else to find some way of obtaining the healing substance in a concentrated form. So far as pneumonia is concerned, neither of these conditions have been fulfilled to an extent analogous to that for tetanus and diphtheria. In fact we do not possess any such satisfactory experimental basis for the adoption of serum therapy in the treatment of human pneumonia, as we do in the case of tetanus, and it is desirable that laboratory work should supply this basis in the case of an infectious disease, before the general introduction of this principle of treatment for that disease. It is entirely comprehensible from what we know as to the nature of passive immunity and the properties of immune serum, upon which blood-serum therapy depends, that there may be no difficulty in obtaining by this method powerful therapeutic effects in small animals, such as mice and rabbits, and yet we may not be able to attain with the same serum any curative effect in a large animal or man. A person may possess blood and fluids of sufficient immunizing power to protect himself and still not furnish serum of sufficient power to cure another person. It remains yet to be demonstrated by a process such as is successful for tetanus, or by any other process, that animals may be rendered so highly immune from pneumococcus infections as to furnish serum curative for human pneumonia.

Whether or not human beings who have recently recovered from an attack of pneumonia or in other ways have acquired immunity from the pneumococcus may yield serum of sufficiently high immunizing power to exert a curative influence in human pneumonia, cannot at present be positively stated. Some observations indicate that the injection of serum obtained under these circumstances may influence favorably the course of the disease, but it does not appear that any such constant and specific therapeutic effects have been attained as to justify sanguine hopes that by this procedure with its present possibilities the treatment of pneumonia is to be revolutionized.

The immunity in man following an attack of pneumonia appears to be variable both in degree and in duration. How long it may last we cannot say, but repeated attacks of this disease are common. In fact it has been held that one attack increases susceptibility to subsequent attacks. These facts would suggest that the protection temporarily afforded by a single attack of pneumonia is not due to the production of a very large amount of immunity substance, but we know too little of the real conditions on which immunity from this disease depends and as to the conditions determining duration of immunity to establish such an inference.

The practical difficulties in the way of obtaining, preserving and applying healing serum are sufficiently obvious to require no especial comment. Inasmuch as the blood of pneumonic patients at and soon after the crisis may, as I have found by experiments on rabbits, possess marked toxic properties, the use of such blood serum cannot be said to be free from possibilities of danger.

The future may succeed in surmounting difficulties which are now apparent. With a clearer understanding of the basis of immunity from the pneumococcus means may be found to heighten this immunity. Methods may be discovered by which curative substances may be separated in a more concentrated, permanent and easily handled form, than that in the original serum of immune individuals.

The present outlook for the successful employment of the direct chemical products of bacteria and more particularly of the active vaccinating products, in the treatment of acute infectious diseases is not promising. Inasmuch as it is by such actively immunizing products that immunity is under natural conditions acquired from an infectious disease, and as we have reason to believe that natural recovery consists essentially in the development of this immune condition, it did not seem unreasonable to hope that the injection of such vaccinating bacterial products might exert a curative influence. It was soon observed, however, that the immediate effect of such injection, corresponding to the period of reaction before establishment of immunity, is often distinctly unfavorable and is attended with increased susceptibility to the microorganism yielding the products.

Whether this unfavorable influence may be checked by further purification of the vaccinating products and removal of toxic substances is not at present clear. Some experiments, especially with the pneumococcus, seem to indicate such a possibility, but it cannot be said that we have yet succeeded in wholly separating the protective from the toxic properties of bacterial products and it may be that retention of some toxic property is essential for the production of immunity.

A further obstacle to the successful application of these vaccinating substances to the treatment of infection, is that unlike the healing serum they require an interval of time, often of days, before insusceptibility to the disease is manifested and hence they cannot be expected to do any good in rapidly fatal infections. By concentration of the vaccinating fluids this interval may be shortened, and in this way Klemperer has succeeded in curing slow pneumococcus infection of moderate severity in rabbits. At the present moment however, hopes of finding specific curative agents rest in healing serum rather than in cultural bacterial products. We are in the midst of a period of active experimentation revealing new and unexpected facts and points of view concerning this whole subject, and it would be hazardous to pass any verdict at the present moment.

We have now passed in review the more important facts concerning the known morphological and biological properties of *Diplococcus pneumoniae.* It now remains to consider the observations relating to the presence and effects of this interesting microorganism in human beings.

That *Diplococcus pneumoniae* is the specific cause of at least the great majority of cases of acute lobar pneumonia is no longer open to dispute. This organism is present regularly and in large number in the pulmonary exudate of this disease, and also in most of the complicating inflammations, such as pleurisy, pericarditis and meningitis. In the majority of cases it is the only bacterial species present. The pathogenic effects obtained by inoculation of its cultures in animals, form an important link in the chain of evidence. The proof is clinched by the demonstration in the bodies of those affected with or convalescent from croupous pneumonia of specific toxic and immunizing proteids, which, so far as known, can be produced only through the activities of the pneumococcus.

The only point in this connection which can be considered at all unsettled, is whether this organism is the specific cause of all cases of genuine acute lobar pneumonia. The pioneer investigators of this subject, A. Fraenkel and Weichselbaum, expressed opposite opinions on this point, Fraenkel believing that the pneumococcus is the sole cause of lobar pneumonia, and Weichselbaum contending that it is the most common and important cause, but that in some cases other organisms play the casual rôle.

The number of competent bacteriologists—and this question is purely a bacteriological one—who hold to Weichselbaum's eclectic view is much smaller now than formerly, and is decreasing with advance in our knowledge of the peculiar properties of *Micrococcus lanceolatus.*

Failures to find this organism in the affected lung in cases of croupous pneumonia date for the most part from a period in which all of the requirements necessary to establish its absence were not completely understood. Ordinarily there is no particular difficulty in demonstrating the presence of the pneumococcus in croupous pneumonia by microscopical examination, by culture and by inoculation of mice and rabbits with a bit of the hepatized lung. But it may happen that one or two of these procedures give a negative result, when the other succeeds. Therefore when all three methods have not been employed, a negative result of the bacteriological examination is not conclusive. At the time of the examination it may be that most of the cocci are dead or incapable of development in our cultures. This is particularly common in old metapneumonic empyaemas.

From what has already been said regarding the assumption by pneumococci of the form of streptococci, it is probable that some investigators have described under the latter name in cases of croupous pneumonia forms of the pneumococcus.

It is not very uncommon to find associated with the pneumococcus in croupous pneumonia other bacteria, particularly the pyogenic staphylococci and streptococci, and less frequently Friedländer's pneumo-bacillus. The coexistence of these other bacteria, although it may influence the course and character of the disease, cannot of course detract from the etiological primacy of the pneumococcus.

It will be remembered that *Micrococcus lanceolatus* was first discovered by Sternberg and by Pasteur in the human saliva, and hence it received the name of micrococcus of sputum septicaemia, as the injection of saliva containing virulent lanceolate cocci causes septicaemia in rabbits and mice. The pneumococcus is a frequent inhabitant of the mouth in health, occurring in the virulent state in this situation in fifteen to twenty per cent of healthy adults. It is even more common in the mouths of those who have recovered from pneumonia. According to Netter it is to be found in sixty-six per cent of such persons. It is less common in the mouths of children, the estimates varying from five to fifteen per cent.

If we include pneumococci with little or no virulence, the percentage of cases in which it is present in the mouths of healthy persons is much greater. In fact Kruse and Pansini bring good evidence to show that the pneumococcus, or some of its varieties, with or without virulence, is a regular inhabitant of the mouth.

Diplococcus pneumoniae is present regularly in the expectoration of acute lobar pneumonia, but its presence is deprived of diagnostic value by the facts just stated.

The presence of the pneumococcus in the mouth in health is not an argument against the pathogenic powers of this organism in man. We have several other examples of the frequent presence of undoubtedly infectious bacteria in the mouth, intestinal canal and other exposed situations in the human body in health. Indeed the existence of the pneumococcus in healthy persons is a help, rather than a hindrance, to our understanding of the etiology of pneumonia. It permits, even compels, us to give due weight to all of the so-called predisposing, secondary or accessory causes of this disease, causes often so manifestly operative that they may seem to be, as they were once believed to be, the efficient causes.

Pneumonia is one of those infectious diseases in which predisposition is an important etiological factor. In what way such predisposing or secondary causes as exposure to cold, season, climate, preexisting disease, alcoholism, old age, injuries to the chest, act in favoring the onset of pneumonia, we do not know. We can imagine that some may enhance the virulence of the pneumococcus, or facilitate its ingress to the deeper air passages, or render the pulmonary tissues less resistant to its invasion and multiplication, or weaken the general insusceptibility of the system. But with our present imperfect knowledge it is not profitable on this occasion to discuss these speculations. Certain diseases, notably measles and perhaps influenza, have been found to be associated with exaltation of virulence of the pneumococci contained in the mouth. We cannot say how often infection resulting in pneumonia is due to pneumococci received from without, as would seem to be the case in some instances of epidemic and contagious pneumonia, or to pneumococci already present in the body.

The distribution of the specific micrococci in cases of croupous pneumonia is subject to considerable variation. In most cases the number of diplococci in the affected part of the lung is large. Living pneumococci are particularly abundant at the margin of an advancing pneumonia, which is evidence that they are not merely secondary settlers in a soil already prepared for them by preexisting disease. They are often contained within leucocytes.

Pneumococci are generally numerous in the fibrinous pseudomembrane on the pleura, which accompanies all croupous pneumonias reaching the surface of the lung. They are usually present in the bronchial lymph glands.

In the majority of cases the cocci are not abundant in the circulating blood. The larger the quantity of blood used for inoculating culture media or animals, the greater the chances are for demonstrating the presence of pneumococci. It is probable that in most, if not all, cases a certain number of cocci get into the blood, but it may be in such small numbers or for such short sojourn that their presence will readily escape detection.

Sometimes the number of cocci found after death in the blood in the heart and vessels is large, exceptionally as large as in pneumococcus septicaemia of the rabbit. Often, although not invariably, this extensive invasion and reproduction of the cocci in the blood corresponds to the so-called asthenic or typhoid pneumonias.

Inasmuch as the pneumococci are not infrequently carried by the circulation in cases of croupous pneumonia, it is not surprising to find them in distant organs, as the spleen, kidneys, liver, joints, bone marrow, brain and meninges. In these situations they may be present without doing demonstrable injury, or they may multiply and cause lesions. It is not rare to find the specific cocci in the intestinal canal, and they may penetrate the bile.

It is a noteworthy fact that the complications of croupous pneumonia, in contradistinction to those of typhoid fever and many other diseases, are, in the majority of instances, referable to the same microorganism which causes the primary disease. Our consideration of the manifold pathogenic possibilities of this variable organism has prepared us for this fact. Complicating pleurisy, pericarditis, meningitis, peritonitis, endocarditis, nephritis, enteritis, parotitis, arthritis may all be due to special secondary localizations of the pneumococcus. In fact each of these and many other local inflammations may be caused by the same organism, independently of the existence of croupous pneumonia as either a primary or secondary affection.

This general rule as to the etiology of complications of pneumonia is subject to many exceptions. As in typhoid fever, scarlatina, diphtheria, tuberculosis, pyogenic staphylococci and streptococci, may find the way open in pneumonia for their entrance and multiplication within the body, and these secondary invaders may cause various local inflammations and septicaemia.

The occurrence of abscess of the lung as a complication or sequel of pneumonia is usually the result of secondary infection with pyogenic cocci. In gangrene of course the putrefactive bacilli are present.

It is interesting to consider what lesions are attributable to the actual presence and multiplication of the pneumococci and what to the absorption of soluble chemical products into the general circulation. In all acute exudative inflammations the cocci are present in the exudate. The diffuse fatty and parenchymatous degenerations are due to the absorption of chemical products. There are probably few at the present day who any longer attribute these degenerations in pneumonia to high temperature. There is evidence that swelling of the spleen, which is common, although not constant, in pneumonia is also due rather to the action of absorbed chemical products than to the actual presence of the microorganism in this organ. Focal necrotic lesions of the liver, such as are extremely common in pneumococcus infections of the rabbit, I have occasionally met with in the human liver in pneumonia. I have experimental evidence that the toxic chemical products of the pneumococcus are capable of producing these focal necroses. To the action of the products of the pneumococcus we can attribute the increase of the fibrin factors in the blood, which characterizes many cases of pneumonia.

The decision as to the relative influence of the actual presence of the pneumococcus, or of the action of its absorbed products in causing the renal complications of pneumonia, is not at present altogether clear. Pneumococci are more frequently, in my experience, to be found in the kidneys in cases of croupous pneumonia than in any other extrathoracic organ. Faulhaber, who has carefully studied this question, thinks that it is improbable that the purely parenchymatous changes, such as desquamation, cloudy swelling, fatty degeneration and necrosis of epithelium, the hyaline degeneration of vessels and the formation of casts are referable directly to the bacteria. These changes are more probably attributable to bacterial products. On the other hand he considers that the inflammatory alterations, particularly the exudation of white and red corpuscles and of fluids and the interstitial infiltration with small round cells are probably directly dependent upon the presence of bacteria in the kidney. Inasmuch, however, as it is possible to produce experimentally by the chemical products the enumerated inflammatory changes, there is no strict proof that these also may not be due to bacterial toxic products. In other words, I think it probable that all of the lesions of an acute diffuse nephritis may be caused by toxic substances, without the necessary presence of bacteria.

Pneumococci have been found only exceptionally in the urine of pneumonic patients.

It has been shown by experiments upon animals that the injection of bacteria or their products into the circulation causes primarily a diminution in the number of leucocytes in the circulating blood. In rapidly fatal infections this diminution is often extreme, and may continue until death. If recovery takes place there is an increase in leucocytes, often considerably beyond the normal. In infections of protracted course there is no general law, often a notable oscillation in the number of leucocytes being observed within short intervals. If the infection is characterized by local inflammatory exudation, there is leucocytosis, proportionate within limits to the extent and character of the inflammation. This leucocytosis is attributed to the absorption by the blood of positively chemotactic substances, such as we know to be frequently formed by bacteria, but it cannot be said that this explanation of leucocytosis is proven beyond question. In accordance with these experimental observations we find that leucocytosis, in greater or less degree, is commonly present in pneumonia. The absence or disappearance of leucocytosis in this disease is an unfavorable prognostic sign.

The pneumococcus has been demonstrated to be the specific cause of all the varying types of genuine acute lobar pneumonia, the sthenic, the asthenic, the bilious, the typhoid, the alcoholic, the epidemic, etc.

We owe to Jürgensen the distinction between primarily and secondarily asthenic pneumonias. So far as the secondarily asthenic pneumonias are concerned, that is, pneumonias which assume an asthenic type as the result of alcoholism, old age, preexisting disease or other debilitating influences, it is not necessary to assume any increased virulence on the part of the infecting pneumococci. The greater gravity of the symptoms can be explained by the lessened resistance of the system to the pneumococcus and its poisons.

There is more reason to assume more than ordinary virulence on the part of the pneumococci in primarily asthenic pneumonias, especially in times and places where nearly all who are attacked, including the previously vigorous, acquire the asthenic type. The extraordinary variability in the degree of virulence manifested by the different varieties of *Diplococcus pneumoniae* renders this view permissible. Nevertheless, even under these conditions, it is possible that the asthenic type is due to influences, which, acting perhaps upon many persons in a household or community, weaken resistance to the pneumococcus.

Although enlightened physicians have often recognized that the gravity of acute lobar pneumonia cannot be measured by the extent of the pulmonary inflammation, nevertheless it is not uncommon to find writers even at the present day who attribute the principal dangers of the disease to such elements as hyperaemia of the lungs, accumulation of inflammatory products, obstacles to the pulmonary circulation, removal of lung surface from the respiratory function. Indications for treatment are sometimes based chiefly upon this mechanical conception of the disease.

The degree and extent of the pulmonary inflammation afford an index of the severity of the disease much in the same way as high temperature does. They are not the real dangers in the majority of cases, although they are of course not to be ignored as sometimes the direct cause of grave symptoms, and as affording indications for treatment. Both clinical experience and the bacteriological study of this disease support the view that in the majority of cases the grave constitutional manifestations of lobar pneumonia are due to toxic substances circulating in the blood and acting injuriously on the respiratory centres and other parts of the nervous system, on the heart, the kidneys and other parts.

There are indeed reasons which lead us to think that local inflammatory exudations in infectious diseases may subserve a useful, conservative, protective purpose. We cannot regard the inflammatory exudate in pneumonia as *per se* a good thing, but it may be the most desirable occurrence possible under the circumstances of the invasion of virulent pneumococci into the lungs of susceptible persons. Experiments upon animals show that it is just those infections with the pneumococcus which are unattended by marked inflammation at the point of entrance which run a rapidly fatal course, and that there is often a direct relation between the development of local inflammation and the severity of the disease in the sense indicated.

Even if it were within our power to arrest the hyperaemia and exudation of leucocytes and other products of inflammation, we should confer a very doubtful benefit upon the patient, unless at the same time we injured the bacteria. It is very likely that in so doing we should rob nature of a weapon which she is using as efficaciously as possible against these invading micro-organisms. It is doubtless often a fortunate thing for the patient that it is not within the power of the physician to accomplish what he considers to be indicated in the way of treatment.

MICROCOCCUS LANCEOLATUS, WITH ESPECIAL REFERENCE TO THE ETIOLOGY OF ACUTE LOBAR PNEUMONIA[1a]

NOMENCLATURE

Various names have been given to the species of bacterium which we believe to be the specific cause of acute lobar pneumonia, such as *Microbe septicémique du salive* (Pasteur), *Micrococcus pasteuri* (Sternberg), *Coccus lanceolatus* (Talamon), coccus of sputum septicaemia (A. Fraenkel), *Bacillus salivarius septicus* (Biondi), *Bacillus septicus sputigenus* (Flügge), diplococcus or diplo-bacillus or pneumococcus or pneumonia coccus of Fraenkel and Weichselbaum, *Diplococcus pneumoniae* (Weichselbaum), meningococcus (Foà and Bordoni-Uffreduzzi), glœococcus of Fraenkel (Klebs), *Micrococcus or Diplococcus lanceolatus capsulates* (Foà and Bordoni-Uffreduzzi), *Streptococcus lanceolatus pasteuri* (Gamaleia), *Micrococcus pneumoniae cruposae* (Sternberg). It is probable that *Micrococcus pyogenes tenuis* of Rosenbach is identical with the pneumonia coccus, as was first pointed out by Neumann.[1] It is evident that this organism has been sufficiently baptized. Of these names, *Micrococcus* or *Diplococcus lanceolatus* (with or without the additional epithet capsulatus), *Diplococcus pneumoniae,* and pneumonia or pneumococcus (Fraenkel-Weichselbaum) have gained the widest currency and will be used in this article. The objection to the names pneumococcus, *Micrococcus pneumoniae cruposae, Diplococcus pneumoniae* is that they suggest an exclusive relationship of the organism to acute croupous pneumonia, whereas this same organism is concerned in the causation of epidemic cerebrospinal meningitis and many other affections independent of pneumonia. Perhaps the name *Micrococcus lanceolatus* is the least objectionable. Certainly one ought to hesitate before adding a new name to the already long and confusing list of synonyms.

[1a] The articles are based upon my lectures on the causation of acute lobar pneumonia, delivered at the Johns Hopkins Hospital in January, February and March, 1892, and upon the presidential address before the Medical and Chirurgical Faculty of the State of Maryland in April, 1892. As the publication of the articles has been delayed, the literature of the subject has been considered up to the present date. I have attempted to present in these papers a summary of the existing knowledge of *Micrococcus lanceolatus,* more especially in its relation to acute lobar pneumonia. My own observations of this organism relate to cultures and results of inoculation from about fifty cases of acute lobar pneumonia, from inflammatory exudates in several cases not associated with pneumonia, and also from the saliva or expectoration of persons not affected with pneumonia. [This article is the first instalment of a series of papers, the latter, however, never having been published.]

Johns Hopkins Hosp. Bull., Balt., 1892, III, 125-139.

History

Micrococcus lanceolatus was discovered by Sternberg in September, 1880, by inoculation of rabbits with his own saliva. It was next found by Pasteur in December, 1880, by inoculating rabbits with the saliva of a child dead of hydrophobia. Pasteur's[2] observations were the first to be made public, being announced at the meeting of the Académie de Médecine in Paris on January 18, 1881. They gave rise to no less than six communications to the same Academy in January, February and March, 1881.[2a] Sternberg's[3] first publication on this subject appeared on April 30, 1881.

[2a] As the exact order in time of these first publications is rarely given correctly it may be well to state their precise sequence:

I. Bulletin de l'Acad. de Méd. Séance du 18 Jan. 1881. Raynaud et Lannelongue. Recherches expér. sur la transmission du virus rabique de l'homme au lapin. In the discussion following the reading of this paper Pasteur said that on December 11, 1880, he had inoculated two rabbits with the saliva of a boy dead, four hours previously, from hydrophobia. In the blood of these rabbits, which died in 36 hours, he found a new species of bacterium, shaped like a figure of 8, surrounded with a gelatiniform envelope. The organism is virulent in broth cultures, in which it often forms chains. He expressed entire ignorance as to the relation of the organism to rabies, but emphasized the distinction between the new disease produced in rabbits and his *Vibrio septique* (Bacillus of malignant oedema).

II. *Ibid.* Séance du 25 Jan. 1881. Pasteur. Note sur la maladie nouvelle provoquée par la salive d'un enfant mort de la rage. This communication was made by Pasteur in the names of himself, Chamberland, Roux and Thuillier. It contains a more detailed account of the new organism and the experiments mentioned at the previous meeting.

III. *Ibid.* Séance du 1 Fév. 1881. A prolonged discussion took place, especially between Pasteur and Colin, as to the propriety of regarding the "nouvelle maladie" of rabbits as septicaemia. The president of the Academy appointed a commission to report on this question.

IV. *Ibid.* Séance du 8 Fév. 1881. Villemin, in the name of this commission, reports that the new disease is not septicaemia (that is, is not the affection then called septicaemia by French authors and which is produced by Pasteur's *Vibrion septique* or the bacillus of malignant oedema).

V. *Ibid.* Séance du 22 Mars, 1881. In a letter to M. Parrot and presented by him to the Academy, Pasteur reports that he has now found the new bacterium in a second case of rabies (Feb. 24), in the mouths of three children dead of broncho-pneumonia and in the saliva of an healthy adult. He failed to find it in the saliva of three adults dead of common diseases. He says that it is evidently

At this time there was no suspicion that this micrococcus of the saliva is concerned in the causation of lobar pneumonia. The bacteriological study of this disease, however, had already begun and went on for a time without any knowledge of the identity of the pneumonia coccus with the organism described by Sternberg and by Pasteur.

The idea that acute lobar pneumonia, at least in some of its forms, is an infectious disease, is centuries old, as has been shown by Hirsch,[4] but this idea gained wider acceptance and the arguments in its support were more fully and forcibly presented in the seventh decade of this century than ever before. Jürgensen,[5] Leichtenstern,[6] and Flint[7] may be mentioned as strong and influential advocates of this view at this period. They brought together the leading anatomical, etiological and clinical facts at that time available to support the doctrine of the infectious nature of lobar pneumonia. The minds of many physicians, therefore, were prepared to accept the discovery of a specific microorganism as the cause of the disease.

The attempts made by Klebs[8] as early as 1875 to cultivate bacteria from cases of croupous pneumonia were not by methods which could yield pure cultures. For this and other reasons his observations concerning his monas pulmonale cannot be utilized.

There can be no doubt that in 1881 Eberth[9] and Koch,[10] and in 1882 Friedländer,[11] Leyden[12] and Günther,[13] demonstrated microscopically, in lungs affected with croupous pneumonia and in other parts of the body, the genuine diplococci of this disease, but they did not cultivate the organism. Leyden and Günther demonstrated the oval diplococci, surrounded with a capsule, according to Günther, in fluid withdrawn during life from hepatized lung.

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a common inhabitant of the mouth and that it has nothing to do with rabies. He believes that this organism will be found to have great significance in the etiology of disease.

VI. *Ibid.* Séance du 29 Mars, 1881. Vulpian reports that he has also produced Pasteur's new disease by the inoculation of rabbits with the saliva of healthy persons. He made known these results in his lectures at the Faculty of Medicine on March 19 and 22, being assisted by Bochefontaine and Arthaud.

VII. Sternberg, National Board of Health Bulletin, April 30, 1881, says that he has described this organism in a report (not then published) made to the National Board of Health in February, 1881. At the time of this publication Sternberg had become familiar with Pasteur's first communications on the "nouvelle maladie." While engaged in experiments intended to control Klebs and Tomassi-Crudeli's statements regarding the so-called *Bacillus malariae*, Sternberg found, in New Orleans, in September, 1880, that the subcutaneous inoculation of his own saliva in rabbits produced a fatal form of septicaemia with the micrococci under consideration in the blood. These first experiments were followed by others in the early part of 1881 in Philadelphia and Baltimore.

Manifestly little importance can be attached to such observations as those of Mátray[14] and of Ziehl[15] in 1883, who examined only microscopically pneumonic sputum, although their descriptions leave little doubt that they saw the pneumonia cocci.

It had been long known that pneumonic sputum may occasionally manifest toxic or infectious properties when inoculated into certain animals, but no specific significance could be attached to these observations, inasmuch as the sputum in other conditions and even in health may possess similar properties. In 1882 Griffini and Cambria[16] produced regularly septicaemia by inoculating rabbits and dogs subcutaneously with pneumonic sputum, and found that the disease could be transmitted by inoculating the blood of the animals dead of the infection.

In October of the same year Salvioli and Zäslein[17] claimed that cocci can be demonstrated regularly in the sputum, blood and blister serum of patients affected with croupous pneumonia and that fluid cultures from the blood or serum of cocci, sometimes mixed with rods, threads and spores, produce typical pneumonia and pleurisy when inoculated in rabbits and white rats. Very little weight can be attached to these experiments of Salvioli and Zäslein.

No little sensation was made by Friedländer's[18] publication in November, 1883, in which he described cultures of his pneumococcus. We now know that the so-called pneumococcus cultivated by Friedländer is an organism entirely distinct from *Micrococcus lanceolatus*, and is to be regarded as a bacillus. This pneumo-bacillus of Friedländer is probably in no way concerned in the causation of genuine acute lobar pneumonia in man. Friedländer's method of cultivation in nutrient gelatine at ordinary temperatures precluded his obtaining the genuine pneumonia coccus. The historical importance of this publication of Friedländer is that he was the first to describe cultures obtained from croupous pneumonia by using Koch's solid nutritive media, that his pneumo-bacillus was widely accepted until the year 1886 as the specific cause of croupous pneumonia, and especially that his results gave an impetus to the bacteriological study of this disease by modern methods.

Inasmuch as Friedländer described his pneumonia organism as an oval coccus with a capsule, and as *Micrococcus lanceolatus* is an oval coccus with a capsule, there followed much confusion of these two organisms, which in fact can be readily differentiated by cultures and by inoculation of animals.

In contradistinction to these observations of Friedländer, those of Talamon,[19] made public before the Société Anatomique in Paris on November 30. 1883, unquestionably pertained to the genuine *Micrococcus lanceolatus*. He recognized microscopically lanceolate cocci as the most common organism in pneumonic sputum and exudate. This species of coccus was present in his bouillon cultures of pneumonic exudate at body temperature, and in two instances in pure cultures. Sixteen out of twenty rabbits inoculated in the chest with these cultures died of septicaemia combined often with fibrous pleurisy and pericarditis, and the blood and exudates of these animals contained the same lanceolate cocci and gave origin to pure cultures which were again inoculated with the same results. Guinea pigs and dogs were described as immune. Talamon found the characteristic cocci in fluid withdrawn during life by a hypodermic syringe from hepatized lung (8 cases), and once from this fluid he obtained a pure culture. Only once did he find the coccus in the blood of the patient during life, and this was at the moment of the death-agony.

Notwithstanding incompleteness and points open to criticism, Talamon's publication is the first in which it is clear that the author has worked with cultures of the genuine *Micrococcus lanceolatus* obtained from lungs affected with croupous pneumonia. The name, *Coccus lanceolatus,* which he gave to the organism is perhaps the best which it has yet received. Some French writers call it the pneumococcus of Talamon, in recognition of his merit in being the first to cultivate it from pneumonia.

It is also clear that in 1884 Salvioli [19a] worked with the lanceolate coccus obtained by inoculating rabbits and guinea pigs with pneumonic sputum and exudate, and with pleuritic and pericarditic exudates from cases of pneumonia.

In April, 1884, A. Fraenkel [20] reported to the Congress für Innere Medicin in Berlin the results of his cultivation on solid media of the pneumonia coccus from the lung in three cases of croupous pneumonia. Unlike Friedländer, he found cultures of this coccus in one case pathogenic for rabbits and he missed the nail-like growth in gelatine. Friedländer pronounced this coccus to be a different organism from that which he had cultivated, and in reply Fraenkel (in a note appended to his report in the Transactions of the Congress) maintains that the organism which he has cultivated is the genuine pneumonia coccus. In this report Fraenkel calls for the first time attention to the similarity between the coccus of sputum septicaemia and that of pneumonia.

In a communication of July 13, 1885, A. Fraenkel [21] describes more fully the coccus of sputum septicaemia, which he now finds in its morphological and biological characteristics to be identical with the pneumonia coccus. The occasional presence of this organism in the mouth in health helps us to comprehend the action of the accessory causes of pneumonia, such as exposure to cold. In this as well as in the previous communication Fraenkel directs attention to the loss of virulence sustained by the pneumonia coccus in the pulmonary exudate in the course of croupous pneumonia.

In a paper read before the Pathological Society in Philadelphia, in April, 1885, and published in July, 1885, Sternberg[22] identified the micrococcus which he had previously found in his saliva, and which A. Fraenkel called the coccus of sputum septicaemia, with the micrococcus which he demonstrated in the sputum and lung of croupous pneumonia. Both Sternberg and Fraenkel, therefore, independently of each other and at about the same time, identified genuine *Diplococcus pneumoniae* with the micrococcus of sputum septicaemia (Sternberg's *Micrococcus pasteuri*), but Sternberg fell into the error, common at the time and which he corrected in July, 1886,[23] of regarding this microorganism as identical with the so-called pneumococcus of Friedländer.

This period of confusion was brought to an end by the exhaustive and fundamental researches of A. Fraenkel, published in 1886, the first article[24] appearing in January, the second[25] in April and the third[26] in November. These articles furnished a full and accurate description of the leading characters of *Micrococcus lanceolatus,* rendered probable its causative relation to acute lobar pneumonia and many of its complications, separated this organism clearly from the pneumo-bacillus of Friedländer, and recognized its identity with the micrococcus of sputum septicaemia.

Not less important were the independent investigations of Weichselbaum,[27] communicated to the Gesellchaft für Aerzte in Vienna in May, 1886, and published in the following October. Fraenkel had cultivated *Micrococcus lanceolatus* from only a small number of cases of croupous pneumonia, whereas Weichselbaum's material consisted of 129 cases of pulmonary inflammation, of which 94 were the genuine lobar pneumonias. He came to the conclusion that the great majority of cases of lobar pneumonia are caused by *Diplococcus pneumoniae,* but that some are caused by Friedländer's pneumo-bacillus or by streptococcus. This eclectic view, which Weichselbaum has never abandoned, is in opposition to the conclusion reached by Fraenkel that all genuine acute lobar pneumonias are caused by *Micrococcus lanceolatus.*

Foà and Bordoni-Uffreduzzi[28] also deserve mention among the pioneer investigators of this period. On March 19 and 26, 1886, they reported to the Royal Academy of Medicine in Turin their observation of lanceolate diplococci in the exudate of epidemic cerebrospinal meningitis. This microorganism, which they called meningococus, they identified with Fraenkel's pneumonia coccus and the coccus of sputum septicaemia, and in the following year they[29] published a valuable study of the biological properties of this organism.

Since these fundamental researches, each year has been rich in contributions to the literature of our subject. These have established firmly the belief in the causation of croupous pneumonia by *Micrococcus lanceolatus,* have extended our knowledge of the morphological and biological characters and especially of the extraordinary variability of this remarkable microorganism, have shown that it is the cause not only of manifold complications of pneumonia but also of many diseases not associated with pneumonia, and have led to interesting discoveries concerning immunity and healing of infections caused by it. Reference will be made to many of these contributions in the course of this article.

MORPHOLOGY

As the name lanceolate coccus implies, the typical form of the pneumonia cocus is oval, with one end somewhat smaller, more tapering than the other. It is often compared in shape to the flame of a candle or to a grain of barley. It is not, however, very uncommon to find, especially in cultures, regularly oval and even spherical forms. The average length of the lanceolate cocci is about 1-1.5μ, and is one-third to one-half greater than the width, but forms occur which are two, three, even four or five times longer than broad. These long forms cannot properly be called cocci; they are actual rods or bacilli. I have met them especially in guinea pigs and mice, and in the subcutaneous exudate in some cases in rabbits. They may also occur in cultures. The propriety of calling this organism a coccus is open to question. Its typical form is transitional between a coccus and bacillus, and the form may be that of an unmistakable bacillus, which is never seen in the genuine micrococci, although the latter may be at times oval. It is called a bacillus by Flügge[30] and Biondi,[31] and in a recent article by Beck,[32] from Koch's Institute for Infectious Diseases, it is called the diplo-bacillus of Fraenkel. I am inclined to the opinion that this organism is more properly designated a bacillus than a coccus, but usage is so overwhelmingly in favor of calling it a coccus that I have preferred to follow the customary nomenclature in this article.

The arrangement in pairs is so characteristic that this has given origin to the name *Diplococcus pneumoniae* or *Diplococcus lanceolatus.* The pointed ends are usually those at the ends of the pair, but the adjacent ends may be pointed. Less common is the biscuit-shape which belongs to the more typical diplococci, such as the gonococcus.

Scarcely less frequent than the arrangement in pairs is that in short chains of four or six cocci. Here the subdivisions into pairs is usually apparent.

Pneumococci may grow also in long and curved chains of cocci. Here, also, the subdivision into pairs and the lanceolate shape may be preserved, or the chains may be indistinguishable from the regular streptococci. This appearance is observed especially in the later generations in liquid cultures. While it is true that the greater tendency to grow in long chains, the less virulent as a rule are the cultures, nevertheless exceptionally the pneumococcus may grow regularly in long chains both in cultures and in the animal body and still equal in virulence the ordinary diplococci, as has been observed by Foà.[33]

Starting with cultures of typical diplococci, I have sometimes obtained in later generations nonvirulent streptococci which I cannot distinguish by culture methods or in any other way from some cultures of *Streptococcus pyogenes.* If, as there is no reason to doubt, the pneumococcus may occur under natural conditions in similar chains and with the same properties, I do not know how under these circumstances it can at present be clearly separated from some other varieties of streptococci. There are streptococcus cultures which die within a week, and others which survive many weeks or months. There is reason to believe that organisms in lobar and other forms of pneumonia have sometimes been described as streptococci without suspicion of their relation to the pneumococcus, while in reality they were varieties of this latter organism.

Streptococci and the various affections caused by them are at present among the most interesting and important objects of bacteriological study. The apparent relationship existing between the pneumococcus and certain kinds of streptococci is a matter of much interest, and it may be expected that future investigations will clear up much of the obscurity at present belonging to this subject.

Even more helpful for diagnosis than the lanceolate shape and the paired arrangement is the presence of a capsule around the cocci, the combination of these three traits having suggested the name *Diplococcus lanceolatus capsulatus,* a designation which is generally appropriate for virulent pneumococci. Capsules are demonstrable around the cocci growing in the tissues and fluids of the animal body. The usual statement is that in most cultures the capsules are not demonstrable. They are, however present often in blood serum, milk and agar cultures, and sometimes in bouillon. Certain varieties present capsules in cultures more regularly and more readily demonstrable than do other varieties of the coccus lanceolatus.

The demonstration of the capsule is in some cases easy, and can be made by ordinary methods of staining; in other cases it is difficult and requires special methods. In difficult cases the following method has given me good results with cover-glass specimens:

These specimens prepared without water, are treated first with glacial acetic acid, which is at once allowed to drain off and is replaced (without washing in water) with aniline-oil gentian-violet solution. The staining solution is repeatedly added to the surface of the cover-glass until all of the acid is displaced. If now the specimen is washed with a saturated aqueous solution of common salt and is examined in this solution, it will be seen that both coccus and capsule are uniformly and deeply stained and cannot be differentiated. If water be used instead of saturated salt solution, the capsules are decolorized, sometimes only in part when they can be clearly recognized, but often completely and they may entirely disappear. By using weaker solutions of salt it is possible in all cases to differentiate the capsule, if it is present. The strength of the solution best adapted to different cases varies. Often the ordinary physiological solution suffices. A generally useful strength is two per cent. The specimens are to be examined in the salt solution. When mounted in balsam the capsules do not always remain distinct. The capsules may appear stained throughout, or only their outer margin may be stained.

This method of staining I worked out on the supposition that the capsules are composed essentially of mucin which is precipitated by acetic acid and, when thus precipitated, is insoluble in concentrated salt solution and swells up in water.

The chief disadvantages of this method are the difficulty of avoiding precipitates of the dye, and the alterations in the cells and other elements, but in difficult cases it will serve a useful turn, although less violent methods when applicable are to be preferred.

The capsules vary in their structure, some being much more delicate and narrower than others. In old exudates the capsules are often very distinct and resistant to the action of water, in which they may appear even quite highly refractive.

Micrococcus lanceolatus stains readily on cover-slip preparations with the ordinary aniline dyes. It is less easily stained in sections. In these it can be stained by Gram's method, but Weigert's fibrin stain gives better results. The number of cocci brought to view by this last method in sections of human lungs affected with lobar pneumonia is often very great, and the presence of the majority of the cocci inside of leucocytes is clearly demonstrated.

Faulhaber,[34] who worked chiefly on the kidneys gives the preference to Löffler's alkaline aniline water dyes, Löffler's alkaline methylene blue solution, and Kühne's carbolic methylene blue solution, in the order named.

Degenerative and involution forms of *Micrococcus lanceolatus* are common both in cultures and in the animal body, especially in old inflammatory exudates. Forms both larger and smaller than normal, pear-shaped, swollen, varicose forms and feebly or irregularly staining or unstained forms, are met with in old cultures and exudates. Arkharow[35] finds that in the blood serum of rabbits rendered immune by the protective vaccination, pneumococci may be present degenerative forms which he describes as short, thin rods staining imperfectly, and often staining more deeply at the slightly swollen ends. A formless detritus may result from the disintegration of the cocci.

Among the most interesting of the degenerative forms are empty capsules and capsules containing faintly staining or unstained cocci, and capsules containing small, often irregular, deeply staining particles which are evidently remnants of cocci. Ziehl[15] was the first to note the occurrence of empty capsules Many of these degenerated capsular forms are not readily made out except by the acetic acid method just described. I[36] described these forms over two years ago as they appear by this method in empyaema. They are to be found abundantly, together with other forms of degeneration, in the exudate of croupous pneumonia, and often in rabbits in inflammatory exudates, especially those of old standing near the seat of subcutaneous inoculation. Sometimes the degenerated cocci are contained chiefly within leucocytes, but they may be abundant outside of the cells. In the later stages of croupous pneumonia it is often astounding what immense numbers of empty capsules or capsules containing dead or degenerating cocci are present, so that the exudate may consist in no inconsiderable part of these degenerated forms.

The sticky, viscid consistence often noticed in inflammatory exudates caused by the lanceolate coccus is probably often due to the abundance of capsules.

Micrococcus lanceolatus is not motile. It never forms spores.

Cultures

Micrococcus lanceolatus grows best at temperatures approaching that of the human body. Below 24° C. (75° F.) the growth may fail, but there may be development at as low a temperature as 18° C. (64.4° F.), especially when the organism has been cultivated for several generations outside of the body. Nutrient gelatine is not a medium adapted for obtaining first cultures of the pneumococcus, as this organism is not sure to grow at temperatures below the melting point of gelatine.

The pneumococcus is capable of growing on all of our ordinary culture media, but there is no other known organism which will grow on so many media and at the same time is so particular as to the exact composition of the medium. Slight differences in the reaction, in the quality of the peptone and in that of the meat often determine whether or not this organism will grow. So difficult is it to control precisely these conditions that one cannot be sure that the nutrient agar which may have been specially prepared is suitable for the development of the pneumococcus until he has tested it. Hence, the absence of any growth of the pneumococcus on agar not thus tested cannot be considered proof that the inoculated material did not contain this organism.

The reaction of the medium should be feebly alkaline. Agar media are best adapted for obtaining pure cultures. Plain nutrient agar, sugar agar and glycerine agar are those generally employed. My preference is for the Guarnieri [37] gelatine agar mixture so modified as to contain more agar than in the original formula and being of a slightly alkaline instead of a neutral reaction.[3a] On this medium the growth is particularly luxuriant.

In agar the deep colonies appear to the naked eye as minute grayish points, the surface colonies as round or slightly irregular, grayish, transparent dots, rarely exceeding 1 to 2 mm. in diameter. Under the microscope the colonies appear finely granular, and according to their thickness have a grayish or more or less of a yellowish hue. In stab cultures the growth is generally more distinct in the line of puncture than on the surface. That this organism may grow in a manner indistinguishable from *Streptococcus pyogenes* has already been mentioned. In such cases the colonies present the coils and peripheral chains seen in streptococcus colonies.

The pneumococcus does not liquefy gelatine. It develops well in alkaline bouillon, producing usually at first a diffuse cloudiness with a small amount of sediment, followed by clearing of the bouillon, but, especially with nonvirulent forms, there may be abundant sediment with or without clouding of the bouillon. The sediment may be pulverulent or flocculent or a uniform mass. There is produced in alkaline media a small quantity of acid.

Milk is usually soured and coagulated by the growth of the pneumococcus. Some varieties, especially nonvirulent ones, do not coagulate milk. There is generally either no visible growth or a faint grayish growth on potato. Varieties, both virulent and nonvirulent, occur which do not grow on solidified calf's blood serum, whereas other varieties may grow well on this substance.

[3a] My modification of Guarnieri's formula is as follows: 950 grm. meat infusion, 5 grm. NaCL, 10 grm. Merck's peptone, 6 to 8 grm. agar, 30 to 40 grm. gelatine. The gelatine and the agar are boiled separately in 50 c. c. water before mixing. The reaction should be distinctly, but feebly, alkaline.

This medium partly melts at 35° C., but colonies in Petri's dishes remain separate and the medium solidifies at room temperatures. It is not adapted for roll cultures.

The lanceolate coccus is a facultative anaerobe, growing without as well as with the presence of free oxygen.

The variability in the properties of this coccus is manifested not less in its behavior in culture media than in other respects, varieties occurring which grow in acid media, which grow well on potato, which do not coagulate milk, which present a more opaque whitish growth than usual, etc. Important modifications in the cultural characters, particularly as to the luxuriance of the growth and the capacity of development at low temperatures, can often be brought about by artificial cultivation. Abundant growth at low temperatures is generally associated with loss of virulence and is often observed in late generations of artificial cultures of primarily virulent pneumococci. Kruse and Pansini,[38] in a recent valuable article, have fully considered the various peculiarities and modifications in the growth of *Micrococcus lanceolatus*.

VIABILITY AND LOSS OF VIRULENCE

One of the most striking properties of the lanceolate coccus both in the animal body and in cultures is its short viability, a property, however, which, like most of the others, is not without exceptions. The maximum development in cultures at body temperature is attained in twenty-four hours or less. After this there is usually a progressive and rapid death of the bacteria, so that they must be planted on new media in larger and larger quantities in order to obtain fresh cultures, and at the end of four to seven days surface agar cultures and bouillon cultures are usually dead. Various circumstances influence the duration of life of the cultures; absence of oxygen, exclusion of light, the proper alkaline reaction, low temperatures, and saprophytic growth for many generations prolonging their vitality. Surface agar and bouillon cultures capable of living two weeks or more are generally not virulent, but there are varieties which retain their virulence even under these circumstances.

In order to be sure of keeping the cultures alive they should be planted over every day or two. But even this precaution will not as a rule preserve the virulence of the organism. After the third or fourth generation it is not uncommon to find diminution of virulence as indicated by the later death of the inoculated animal, and frequently after about ten generations, and sometimes sooner, nearly all virulence has disappeared, although there are varieties which preserve some virulence for at least one hundred generations. In order to keep up the virulence of the cultures the plan is usually adopted of transmitting them frequently through rabbits. In my experience even this will not always succeed.

Kruse and Pansini[38] found living and virulent pneumococci in a serous pleuritic exudate which had been preserved in a sealed glass tube for over a year. Foà[39] recommends the following method for preserving the virulence of pneumococci for a long time and for obtaining material of uniform virulence. The blood of a rabbit which has died of pneumococcus septicaemia of the oedematous variety is collected immediately after death in a sterilized tube, and is put for 24 hours in a thermostat at 33°-35° C. The tube is then sealed and kept excluded from the light. In this way the virulence can be preserved for 45 days. Arkharow[35] has also found that blood withdrawn from the heart of a rabbit dead of pneumococcus infection, preserves its virulence for three to four weeks if it is kept at ordinary temperatures in sealed tubes away from the light.

Monti[40] made the interesting observation, which merits further investigation, that the impaired virulence of *Diplococcus lanceolatus* can be restored by injecting with the cultures into rabbits the products of *Bacillus proteus vulgaris* or other common putrefactive bacteria. Foà[39] found also, that the special characters of the organism were modified by the presence of some other bacteria. His meningococcus, that is the diplocccus of the fibrinogenic variety, was transformed into his pneumococcus, that is the diplococcus of the oedematogenic variety, by inoculating rabbits with a mixture of the meningococcus and *Proteus vulgaris*.

The short viability and rapid loss of virulence give to *Micrococcus lanceolatus* a quite exceptional position among pathogenic bacteria, and they constitute an annoying obstacle to systematic work with this organism, especially to experiments requiring varieties possessing maximum virulence.

The limited duration of life of *Micrococcus lanceolatus* is a matter of great practical interest. It is generally capable of longer life in the virulent state in susceptible animals than in artificial cultures, but it is apt to die even in the living body sooner than most other pathogenic bacteria. Mention has already been made of the large number of dead pneumococci often found in empyaemas, in the exudate of croupous pneumonia, and in local subcutaneous exudates in rabbits. This short vitality is doubtless one reason why empyaemas and other local inflammations caused by the lanceolate coccus usually afforded a more favorable prognosis than those caused by streptococci, and why a single aspiration of the pneumococcus empyaema may be followed by a permanent cure. But here, as well as with other properties of this peculiar microorganism, it is necessary to keep in mind the frequent exceptions to the rule, instances occurring in which virulent pneumococci are found in empyaemas and other inflammatory exudates at least six months old.

It was stated by Fraenkel[24] and Weichselbaum[27] and the statement has been confirmed by Netter,[41] Monti,[42] Banti,[43] Patella[44] and others, that the pneumonia coccus suffers a progressive loss of virulence in the pulmonary exudate in the course of croupous pneumonia. Patella and others determined this by withdrawing with a hypodermic syringe some of the juice of the affected lung at different stages of the disease and inoculating animals. It is asserted by Netter that the expectoration on the day of the crises often loses the virulence which it has previously manifested and remains inactive for the following two or three weeks, although it contains living diplococci which subsequently regain their pathogenic power.

It can scarcely be doubted that attenuation of virulence does occur in the human body, inasmuch as pneumococci obtained from inflammatory exudates may be devoid of any pathogenic effect in animals, but I do not think that we are at present justified in accepting such definite laws regulating this loss of virulence as those laid down by Patella and by Netter. I have obtained from late stages of pneumonia which has gone on to recovery, and at the time of crises, pneumococci which are as virulent and sometimes more virulent than those procured from earlier stages in the same case. In experiments of this character it is important to take into consideration the number and character of living pneumococci inoculated. We know that the pulmonary exudate contains many dead cocci, and we also know that the cocci present may vary among themselves in degree of virulence. Moreover, when we inoculate animals directly with material containing pneumococci from the animal body we may introduce with the organisms toxic, immunizing and healing substances which influence the course of the infection. It will be shown later in this article that such material, although proven to contain very virulent pneumococci, may not produce any infection. These considerations are calculated to cast doubt upon many of the experiments intended to demonstrate loss of virulence in such material as pneumonic sputum and exudate.

There is a wide divergence of statement regarding the influence of desiccation on *Diplococcus lanceolatus*,[4a] a divergence to be explained, according to Kruse and Pansini,[38] in large part by the characters of the particular variety used by the experimenter. Pneumonia cocci in blood and sputum are in general tolerably resistant to drying, living and retaining their virulence sometimes for four months, although they may die much sooner. Pneumococci in thin fluids like bouillon and water are less resistant to desiccation than those in animal juices. It has not been determined whether pneumococci in sputum or blood which has been dried and reduced to a dust-like powder capable of being carried by ordinary air currents can still live and be virulent. The preservation of virulent pneumococci in dried sputum for as long a time as is shown in some of the experiments, indicates the propriety of destroying or disinfecting pneumonic sputa.

[4a] The following are the statements of different experimenters regarding the resistance of the pneumococcus to drying:

Biondi[31] (1887) impregnated sterilized silk threads in young bouillon cultures and dried them at temperatures from 18° to 35° C. The bacteria were dead in 24 hours, as tested on gelatine. Only in one instance was there trace of growth at this period. The dried silk threads were innocuous when placed, even in large amount, under the skin of rabbits at the end of 24 hours.

Foà and Bordoni-Uffreduzzi[29] (1887) found the diplococci both in cultures and in blood dried between watch-glasses at ordinary temperature away from the daylight living and virulent up to the 45th day.

A. Fraenkel[21] found that the pneumonia coccus would not grow at temperatures exceeding about 42.5° C. (108.5° F.), and that cultures at 41°-42° C. (105.8° F.-107.6° F.) rapidly lose their virulence.

According to Sternberg,[50] the pneumonia coccus is killed by exposure for ten minutes to a temperature of 52° C. (125.6° F.). It is not killed by freezing. The action of sunlight is injurious to this as well as to other bacteria.

Pathogenic Effects in Animals

The pathogenic properties of *Micrococcus lanceolatus* are of course those which most directly concern us. As regards these properties the variability of this microorganism is most strikingly manifested. It is an organism of the most manifold and varied pathogenic possibilities, and one must have had

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Guarnieri[37] (1888) found both diffuse and direct sunlight, especially the latter, injurious to the pneumococcus cultures. Blood and sputum are more resistant to drying than are cultures. Loss of virulence is less by rapid than by slow desiccation. A rabbit inoculated with a feather dipped in blood rich in diplococci and kept for 4 months in the exsiccator at 37° C., died after 12 days with characteristic septicaemia.

Netter[45] (1888) used silk threads impregnated with blood, fragments of spleen and pneumonic sputum, and found the organisms after 32 days' desiccation still virulent.

Patella[44] (1888-89) finds, in opposition to Guarnieri, that rapid drying at 38° C. of blood containing diplococci quickly kills the bacteria, while slow drying at low temperatures preserves the virulence a long time.

Nikiforoff[46] (1890) studied a lanceolate micrococcus isolated by Gaffky from influenza pneumonia and which he considered to be distinguished from the pneumococcus, only by longer preservation of virulence and life and by failure to coagulate milk. These points, however, do not suffice to make such a distinction, as they may all belong to undoubted pneumococci. Nikiforoff's diplococcus, which was undoubtedly a variety of *Diplococcus pneumoniae*, preserved its vitality and virulence for five weeks when dried on silk threads and a very large experience with it before he will have exhausted these possibilities. It is the cause in human beings of a large number of affections hitherto regarded as without any etiological relationship with each other. Before considering these it is desirable to learn the experimental results obtained by the inoculation of animals.

We meet under natural conditions and we can readily obtain by artificial cultivation pneumococci totally devoid of any virulence, and at the other extreme we find pneumococci capable of killing rabbits by septicaemia in less than twenty-four hours. Between these extremes occur pneumococci of all possible degrees of virulence and capable of causing manifold pathological lesions.

The effects resulting from inoculation of *Micrococcus lanceolatus* vary with the animal, with the number of bacteria, with the degree and kind of virulence of the bacteria, and with the site of the inoculation. The presence in the material inoculated of other bacteria also, as well as of their chemical products and of other chemical substances, especially such as are derived

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kept in the exsiccator. How much longer the dried organism survived is not stated. Its original virulence was feeble.

Nissen[47] (1891) used silk threads impregnated with twenty-four hour bouillon cultures and dried in the exsiccator containing sulphuric acid. The bacteria were dead in two days and sometimes in one day. Blood containing diplococci and dried in thin layers was without infectious property after five days.

Bordoni-Uffreduzzi[48] (1891) dried small lumps of pneumonic sputum, proven to contain highly virulent pneumococci, on linen cloths at room temperature both by exposure to the air of the room with diffuse daylight and by exposure to the direct rays of the sun. The sputum after 55 days' drying exposed to diffuse light was infectious, but not after 60 days. The sputum dried by exposure to the direct rays of the sun was infectious after 12 hours; how much longer was not determined.

Sirena and Alessi[49] (1892) used silk threads impregnated with bouillon cultures or suspensions in water, and tested various methods of drying and exposure over different drying substances. They found the diplococci dead after exposure in test tubes to direct sunlight in 3 days, after simply hanging the threads in sunlight in 17 days, after drying over calcium chloride in 31 days, over sulphuric acid in 114 days, in thermostat at 37° C. in 131 days, after keeping in dry place in the dark in 146 days, in a moist place in 192 days.

Kruse and Pansini[38] (1892) dried blood from rabbits dead of pneumococcus septicaemia in thin layer on glass plates both at ordinary temperature and in the thermostat and then kept it at 18° C. Tested by means of agar plates, one variety of pneumococcus was dead after two days, two varieties after ten days, four varieties between eighteen days and three months, and two varieties after two to five months, without reference to the method of drying. (The term variety is used here simply to indicate that the cultures were from different sources originally.) The two most resistant varieties grew luxuriantly in gelatine and were of slight virulence.

from the pneumococci themselves, may likewise influence the course and the character of the infection.

Rabbits and mice are the animals usually selected for inoculation. Mice are more certain to die than rabbits, and should therefore be used when it is desired to determine by inoculation the presence or absence of pneumococci in suspected material. I have, however, met a few cases in which mice survived and rabbits were killed by inoculation with the same exudate from croupous pneumonia, and this result could not be explained by the use of relatively larger quantity of material for the rabbit. (See Appendix, Case C.) It is well, therefore, to inoculate both a rabbit and a mouse with suspected material. Rabbits are much more suitable than mice for the study of the pathological lesions produced by *Micrococcus lanceolatus.* Young animals are more susceptible than old ones and, especially as regards the less virulent varieties of pneumococci, there are differences in susceptibility even between rabbits of the same age and size. The degree of resistance of the individual animal is therefore a factor of some importance.

Guinea pigs and dogs are much less susceptible than mice and rabbits to pneumococcus infection, requiring larger doses and more virulent cultures to kill them. Kruse and Pansini[88] find that dogs are more readily killed by subcutaneous inoculation of pneumococcus cultures, than by intravenous, intratracheal or intrathoracic inoculation of even larger quantities, whereas, as has been long known, in guinea pigs intravenous and intraperitoneal inoculations are more effective than subcutaneous ones. These animals are less prone than rabbits to develop abscesses from subcutaneous inoculations of this organism.

Rats and cats are relatively resistant, but can be infected by large doses of virulent cultures. The same is true of sheep.

Chickens and pigeons are insusceptible to even very large doses.

It is easy to demonstrate that the pathogenic effects may vary greatly according to the number of pneumococci inoculated. The three principal types of pneumococcus infection according to degree of virulence, viz., acute septicaemia, spreading inflammatory exudations and circumscribed abscess, may be produced sometimes by the same culture inoculated into rabbits in varying quantities. Here also the differences are more readily brought out by the less virulent cultures, those of extreme virulence causing septicaemia regularly, even in minute doses. When the virulence is much weakened small quantities may produce no appreciable effect, and larger quantities may kill. It is customary to use rather large doses, often as much as 1 c. c. and more of fresh bouillon cultures, for ordinary inoculation with the pneumococcus.

In making comparative tests of the virulence of different cultures it is of course necessary that the conditions of the experiments should be as nearly identical as possible as regards the age of cultures, the quantity inoculated, the site of inoculation, the size of the animal, etc. As a rule, subcutaneous injections of bouillon cultures or uniform suspensions of agar cultures which are eighteen to twenty-four hours old in the thermostat, are to be preferred. A principal point in determining the degree of virulence is the duration of life of the inoculated animal. When several cultures as tested with the ordinary large doses appear to be of equal virulence that possessed of the greatest virulence is determined by fixing the minimal dose which will kill.

We must recognize, I believe, variations not only in the degree but also in the kind of virulence. We meet with varieties of the pneumococcus which produce regularly difinite pathological lesions in rabbits, and other varieties which cause uniformly different lesions, although one may kill as quickly as the other, and the conditions of the experiment as regards the animal, the age of culture, the quantity inoculated and the site of inoculation may be identical. These differences in quality of virulence may be transmitted to successive generations of the organism although they are frequently inconstant. While recognizing in principle variations in quality of virulence, it is by no means easy in all cases to determine to what extent different pathogenic effects depend upon differences in quantity or in quality of virulence.

Injections of virulent pneumococci into the circulation or into the peritoneal cavity of rabbits generally kill sooner than subcutaneous inoculation, and may cause septicaemia when the latter causes chiefly spreading inflammations. On the other hand, subcutaneous injections of weakened cultures may be followed by abscess formation when intravenous inoculations are without effect. Variations in the type of septicaemia may sometimes be brought out by varying the seat of inoculation. Thus as Foà [39] has shown, the same variety of *Micrococcus lanceolatus* which produces regularly the form of septicaemia characterized by marked subcutaneous oedema and only slight or moderate enlargement of the spleen without increased consistency of this organ, may, when injected directly into the circulation, cause septicaemia with large, hard, fibrinous spleen.

The results obtained by inoculations intended to bring out certain special localizations of pneumococcus infection, such as intracranial, intratracheal, intrathoracic, intraarticular, inoculations, will be described later.

There are sometimes striking differences in the results obtained by inoculating with material taken directly from the animal body, and with cultures of pneumococci isolated from the same material. Thus, inoculation with pneumonic sputum, pneumonic exudate, other pneumococcus exudates, the blood of rabbits dead of pneumococcus infection, may yield results different from those observed after inoculation of pure cultures from the same sources. There are various circumstances, not all of which are well understood, which appear to influence these results.

Material from the animal body, particularly old inflammatory exudates are likely to contain degenerating and dead pneumococci, whereas fresh cultures eighteen to twenty-four hours old contain mostly living organisms. It has been demonstrated by making cultures from different colonies growing in plate cultures from pneumonic sputum or pneumonic exudate, that pneumococci of different degrees and of different kinds of virulence are often mixed together in such material. That in the course of croupous pneumonia and other pneumococcus infections the primary virulence may be weakened has already been mentioned. It has furthermore been shown that during pneumococcus infections there are developed in the body, toxic, immunizing and healing substances, and these may be inoculated with the microorganisms and modify in various ways the course of the infection or arrest it altogether, or cause death by intoxication without multiplication of the organisms.

It is evident from the foregoing considerations that there are many factors which influence the result of inoculation with material containing *Micrococcus lanceolatus.* It is evident that we must not lose sight of these various factors in drawing conclusions as to the degree and kind of virulence possessed by *Micrococci lanceolati* from different sources.

The principal symptoms which may be observed in rabbits inoculated with virulent *Micrococci lanceolati* are fever, diarrhoea and occasionally albuminuria. The temperature is elevated often in a few hours after subcutaneous inoculation and before the appearance of cocci in the circulation. Indeed, even in acute septicaemias where many cocci are found in the blood after death, the organisms can be demonstrated in the circulation often only a few hours before death. They may, however, be present in the blood as long as forty-eight hours before death. Diarrhoea may occur, but it is far from constant. An excess of albumen in the urine beyond physiological limits is a less common symptom, but I have repeatedly noted it. The general condition of the animal is usually surprisingly good until within a few hours of death. At the last the animal becomes weak and stupid. The head droops. Convulsive seizures are not uncommon shortly before death. The animal may cry out. Pulmonary oedema sometimes accompanies the death-agony and may be manifested by frothy serum around the nostrils. In subacute and chronic cases there is loss of weight and there may be profound anaemia. With inflammatory exudates leucocytosis is present.

The lesions found post mortem in rabbits are many and vary with the type of the infection.

Septicaemia is regarded as the leading type of pneumococcus infection. With this the rabbit may die in 16 to 24 hours, but often lives from 48 hours to 4 days. It is possible, therefore to distinguish peracute, acute and subacute pneumococcus septicaemia. If death takes place after four or five days there will generally be found spreading inflammatory exudations or abscess formations, with few or no bacteria in the blood.

Before considering the different types of septicaemia caused by *Micrococcus lanceolatus,* the lesions which may be found in different parts of the body will be described. This description applies chiefly to rabbits.

If the inoculation be subcutaneous, local reaction at this point is rarely entirely absent. It may be predominantly serous or fibrinous, or haemorrhagic or necrotic or purulent. Frequently there are combinations such as fibrinopurulent, fibrinoserous, serohaemorrhagic. The longer the duration of the disease the greater the tendency to fibrinous and to purulent exudations. The extent of reaction varies. It may be limited to the immediate seat of inoculation or, if serous or fibrinous, it may extend over a large part of the body. Frequently at the immediate seat of inoculation there is an opaque exudate of fibrin and leucocytes or of pus, and around this a spreading oedema. Ecchymoses and larger haemorrhages are common. The exudation may extend in depth from the epidermis to the muscles, or to the serous membrane if the inoculation be in the abdominal or thoracic subcutaneous tissue. There may be coagulation necrosis with much nuclear fragmentation of the tissue cells. The lymphatics may be plugged with inflammatory products.

The subcutaneous lymphatic glands, particularly those in communication through lymphatic channals with the seat of inoculation, are often swollen and hyperaemic or even haemorrhagic. They may contain small whitish spots which under the microscope show necrosis with nuclear fragmentation combined usually with an accumulation of leucocytes and sometimes of fibrin. The lymphatic glands may also be swollen and pale, or red and not swollen, or they may be unaltered.

There are sometimes seen in the cutaneous and subcutaneous tissues of the trunk, particularly on the dorsal aspect, scattered, irregular, opaque whitish or reddish-white, circumscribed areas two to ten mm. in diameter, which correspond to spots where the cutaneous striated muscle has undergone necrosis with fatty and hyaline (pseudowaxy) degeneration and often marked vacuolization and disintegration of the fibres. These areas usually contain extravasated blood, especially in the periphery. Similar alterations may be found in the voluntary muscles of the extremities and elsewhere, and occur after intravenous as well as after subcutaneous inoculations.

The spleen may be normal in size and appearance. Usually it is swollen. This enlargement may be moderate or excessive. As regards consistence, two types are observed, the hard and the soft spleens. The typical hard spleens may be dark red, almost black, or they may be light red or mottled. They are dry on section, suggesting sometimes in appearance haemorrhagic infarction. They contain a great quantity of fibrin, which can be well demonstrated by Weigert's fibrin stain, and a large amount of blood. They show more or less fragmentation of the nuclei. The soft spleens do not contain an excess of fibrin. They are rich in blood and often contain blood pigment. There may be no change in the consistence of the spleen.

The liver often contains in variable number, small, opaque, whitish or yellowish-white dots of necrosis. This is in my experience a frequent lesion of septicaemia caused by *Micrococcus lanceolatus,* and it is curious that its frequency and generally its existence should have been overlooked by previous observers. The necroses are usually visible as whitish dots and streaks about 1 to 2 mm. in diameter, most readily seen on the surface, but present also in the depth of the liver. There may be discovered microscopically, when not apparent to the naked eye. They cannot readily be mistaken for parasitic nodules often seen in the rabbit's liver. In the necrotic foci the nuclei of the liver cells are in large part absent or fragmented, the cell body becomes more opaque and refractive than normal and may contain fatty globules and may have a reticulated structure suggesting fibrin, but without the reaction of fibrin. Polymorphonuclear leucocytes wander in from the edges, the liver cells gradually diminish in size or break up and disappear, leaving a peculiar network of intervening glistening lines. Similar foci of hepatic necrosis occur in typhoid fever, hog cholera and many other bacterial infections. I have found them in the liver of human beings dead of croupous pneumonia and in many other infectious diseases in human beings. It has been shown by Dr. Flexner and myself [61] that the presence of bacteria in the liver is not essential to their production, but that they may be caused by the toxic products of bacteria. Cloudy swelling and fatty degeneration of the hepatic cells are common.

The kidneys show often no especial lesion. The renal epithelium may be more or less fatty, and fatty globules may be seen in the glomeruli. The most interesting change observed in the kidney is the presence of hyaline material in the glomerular and intertubular capillaries. This lesion is common in certain types of pneumococcus septicaemia and rarely occurs in others. The hyaline material stains deeply with Weigert's fibrin stain and I regard it as a form of fibrin. It seems to me to be within the capillaries and often plugs them completely. It has been usually described as situated in the perivascular spaces. This capillary thrombosis may be so extensive as to prevent any artificial injection of fluid into the blood vessels of the kidney. In such cases there is almost complete anuria. Similar capillary thrombosis, may be met with in other parts of the body.

The heart muscle is in nearly all cases the seat of fatty degeneration and often extremely fatty. In septicaemias terminating within 18 or 20 hours there may be no fatty degeneration of the myocardium.

The blood is usually found coagulated in the heart and vessels. The right ventricle is often greatly distended with coagula, which may be partly decolorized. The blood may be fluid and the right ventricle small. Leucocytosis is common, and the increase is chiefly of the leucocytes with amphophilic granulation.

Redness of the tracheal mucous membrane and pulmonary oedema are sometimes observed. The lungs may be normal or may present ecchymoses or, especially in cases of long duration, may contain smaller or larger areas of hepatization.

Swelling and hyperaemia of the thyroid and thymus glands are occasionally seen.

Oedema of the mediastinal and subperitoneal tissues may occur. There may be increase of serum in the peritoneal and other serous cavities.

Inflammation of one or more serous membranes is common in infections with certain varieties of *Micrococcus lanceolatus* and is rarely or never observed with others. When it occurs the affection is usually of several days' duration, but multiple serositis may be observed in septicaemias not more than 48 hours in duration. Peritonitis is the most common after subcutaneous or intravenous inoculations, but there may be pleuritis, pericarditis and peritonitis together.

The intestinal contents may be unchanged or they may be softer than normal. Haemorrhages may occur in the intestinal coats, and I have seen necrotic and diphtheritic patches of inflammation in the caecum and colon. The same may also, although very rarely, occur in the mucous membrane of the gall bladder.

Semipurulent exudations in the joints have been noted in some cases.

Subserous, particularly subperitoneal, ecchymoses are common. An infrequent type is the markedly haemorrhagic septicaemia with haemorrahges, sometimes extensive, in the subcutaneous tissue, lymphatic glands and many or most of the organs of the body.

In septicaemia pneumococcica capsulated diplococci are abundant in the serous and inflammatory exudates, if these are present, and are usually abundant in the blood and organs. There may, however, be few cocci in the blood, even in rapidly fatal cases. In cases lasting more than 48 hours the number of cocci in the subcutaneous exudate may be smaller than in the blood and in exudates in the serous cavities. In such cases the subcutaneous exudate usually contains many degenerated cocci, such as have been already described. The diplococci are usually free, but may be within leucocytes, especially in inflammatory exudates. I have seen in a case of multiple serositis most of the diplococci in the pleural exudate within leucocytes, and in the peritoneal exudate extracellular.

It is apparent from the foregoing description that there is much variety in the lesions of pneumococcus septicaemia, cases occurring in which there are scarcely any lesions, and other cases in which there are many localizations of the morbid process. The alterations which may occur in one and the same organ, as the spleen, differ widely in different cases.

The attempt has been made to establish distinct varieties of *Micrococcus lanceolatus* on the basis of these diverse pathological effects. The usual statement however, is that these differences are inconstant, that they depend upon the variations in the degree rather than in the kind of virulence of the coccus, that one and the same variety of lanceolate coccus may produce now one and now another type of septicaemia.

Those who attempt to distinguish distinct biological varieties of the lanceolate coccus do not agree with each other in their classification. Banti[52] makes four varieties and considers that these are only a part of the whole number. His researches are valuable, but his classification has not been confirmed and will not be considered here. More importance in my opinion is to be attached to the distinctions made by Foà and his collaborators in various publications.[53] Foà distinguished originally his meningococcus from the pneumococcus, and even suggested that they were perhaps two distinct species of bacteria. He has modified in several particulars his earlier statements on this point, and only his later views need be here presented. The variety to which he and Bordoni-Uffreduzzi first gave the name of meningococcus he now prefers to call *Diplococcus lanceolatus* of the fibrinogenic (or parasitic) variety, and the second variety, his pneumococcus, he calls *Diplococcus lanceolatus* of the oedematogenic (or toxic) variety. Both varieties may occur alone or mixed in the lung in croupous pneumonia, but the oedematogenic variety is the more common in this situation. He has hitherto met in the exudate of epidemic cerebrospinal meningitis only the fibrinogenic variety. The fibrinogenic variety is characterized by the rapid invasion and multiplication of the cocci in the blood (hence also parasitic variety), and especially by the firm, fibrinous spleen, and fibrinous thrombi (hyaline) in the kidney and elsewhere. The chief characteristic of this variety is the separation of fibrin in the blood. It does not produce local oedema. On the other hand, the oedematogenic variety is characterized by slower multiplication and this chiefly after subcutaneous inoculation in the subcutaneous tissue, where it produces often widely spreading oedema. There are relatively few cocci in the blood, the spleen is soft and contains disintegrated red blood corpuscles, there are no fibrinous thrombi (hyaline) in the kidney or elsewhere. This variety he also calls toxic. Foà now admits that in addition to the fixed types of these two varieties there occur transitional forms in which the type is not fixed and which may produce lesions belonging to both varieties. Pneumonic sputum and exudate may contain both varieties, and when inoculated into rabbits produce both oedematous and fibrinous lesions. The differences between the oedematogenic and the fibrinogenic varieties he says, cannot be attributed merely to differences in degree of virulence, as one may kill as surely and as speedily as the other. They correspond therefore to different biological attributes, to different qualities of virulence. The oedematogenic variety, when injected directly into the circulation, often, but not invariably, produces hard fibrinous spleens, and even after subcutaneous inoculation it may do so if the the fibrin factors in the blood be increased, as by pregnancy, but it preserves its specific properties when introduced subsequently under the usual conditions into the subcutaneous tissue of rabbits. When cultivated anaerobically it can be made to assume the properties of the fibrinogenic variety. This latter variety can be transformed into the oedematogenic when inoculated in combination with certain other kinds of bacteria, particularly *Proteus vulgaris*.

I have met varieties of *Micrococcus lanceolatus* which correspond to Foà's classification, but I am not able to draw the lines of separation as sharply as he does, as in my experience type with inconstant or mixed characters are common. I have, however, obtained cultures which, when injected subcutaneously, produces uniformly extensive local subcutaneous oedema with soft or unchanged spleen, and relatively few organisms in the blood. I have inoculated in series twelve rabbits, varying the dose so that death occurred in sixteen hours up to four days, and have seen no change in the type of septicaemia, especially no hard or fibrinous spleen. This, therefore, corresponds to Foà's oedematogenic variety of fixed type. I have also obtained cultures which produced after subcutaneous inoculation uniformly hard, firm spleens. These differences could not be explained by diversities in the degree of virulence, as with each variety the results were the same after death in 24 hours or 4 days. We must recognize, therefore, varieties of *Micrococcus lanceolatus* with different biological attributes. I have also obtained from single colonies pure cultures which produced a mixed type of septicaemia, that is, subcutaneous oedema with hard fibrinous spleen, with or without an abundance of cocci in the blood, or combined fibrinous and serous subcutaneous exudation with varying character of spleen. I have repeatedly found that cultures or direct inoculations from a rabbit dead of one type of septicaemia produced in a second rabbit the other type of septicaemia. The occurrence of fibrinous (hyaline) thrombi in the kidney is not necessarily associated with the hard, fibrinous spleens; one may be present in the absence of the other, especially may the change in the kidney be absent when the spleen is firm and rich in fibrin.

The presence in the blood of very large numbers of the diplococci is not necessarily associated with a hard, fibrinous spleen. I have found the blood swarming with typical capsulated diplococci without any change in the consistence or size of the spleen, as well as with soft and hard spleens.

The changes in the heart, liver and lymphatic glands above described are common to all varieties of septicaemia produced by *Micrococcus lanceolatus.*

The pathological effects resulting from inoculation of *Micrococcus lanceolatus* depends, as has already been urged, upon many factors, of which differences in degree and kind of virulence are among the most important.

A second type of pneumococcus infection in rabbits may be conveniently distinguished, although it cannot be sharply separated from the septicaemic type. This second type, which is common, is characterized by spreading, localized, usually multiple, serofibrinous or fibrinopurulent inflammations without many cocci in the blood. There is evidence that this exudative type depends upon weakened virulence of this cocci. Rabbits affected with this form of the disease live usually from five to fifteen days and sometimes longer. Pneumococci which at first are of sufficient virulence to produce acute septicaemia, are prone to cause this exudative type when their virulence is weakened, and on the other hand by the passage of the organism through the rabbit the septic type can sometimes be established. Very virulent pneumococci may produce this exudative type in rabbits rendered partly immune against infection.

The subcutaneous tissues for a wide distance around the point of inoculation are thickened and indurated by infiltration with a dense exudate of fibrin, leucocytes and more or less serum, associated often with extravasated blood and with coagulation necrosis of the tissues. In the outlying parts there is inflammatory oedema, sometimes haemorrhagic in character. If the inoculation has been in the subcutaneous tissue of the abdominal wall the exudation may spread over the corresponding side of the abdomen and thorax, over to the opposite side and down the thigh. It may involve the cutis and the intermuscular septa down to the serous membrane. In rare instances this spreading subcutaneous fibrinopurulent exudate is the sole or principal pathological lesion, and it may occur without any bacteria in the blood or internal organs at the time of death.

In the majority of cases there are associated with the subcutaneous exudation similar fibrinopurulent inflammations of the serous membranes, oftenest of the peritoneum, but not infrequently also of the pleurae and pericardium. There may even be pneumonic areas.

The spleen is often little or not at all changed. Large, hard, fibrinous spleens belong rather to the septicaemic type, but there is no relation between the size of the spleen and the number of cocci present in it and in the blood. Indeed, the small spleens in the septicaemic type are often notably rich in cocci.

Foci of hepatic necrosis and fatty degeneration of the heart occur in this as well as in the preceding type of infection.

When the disease is of long duration the animal becomes notably emaciated and anaemic. There is commonly marked increase in the number of leucocytes.

When this second type of the disease is outspoken, there are few cocci in the blood, so few often as not to be readily demonstrable on cover-glass preparations, although demonstrable by cultures. On the other hand, the local exudates contain the characteristic cocci often in enormous number. The cocci may, however, be relatively few or even absent in the subcutaneous exudate which contains many degenerated forms. These forms may be seen also in other exudates.

In this exudative and also in the septicaemic types diplococci may be found in the bile and in the intestinal contents. They are absent from the urine, except in some cases with focal lesions of the kidney due to the presence of the cocci.

No sharp line of separation can be drawn between the first and second types of pneumococcus infection. All grades of transition between them occur and mixed types are common. As has already been mentioned, the differences between these two types depend rather upon the degree than upon peculiarity in the quality of virulence.

In this connection it may be noted that croupous pneumonia in human beings belongs to the exudative rather than the septicaemic type of pneumococcus infection. Inasmuch as the occurrence of such spreading inflammations with little or no invasion of the blood by pneumococci is experimentally shown to depend chiefly upon the relation existing between the virulence of the coccus and the resistance of the animal, and as we cannot suppose that the pneumococci which cause croupous pneumonia are of weakened virulence the inference is drawn that man ranks among those relatively insusceptible to this organism.

A third type of disease caused by subcutaneous inoculation of *Diplococcus pneumoniae* is the formation of an abscess at the point of inoculation. This occurrence indicates either still less virulence than in the preceding types, or, with the same virulence, greater resistance in the part of the animal. Rabbits which have been rendered sufficiently immune to be protected against septicaemia after inoculation with virulent diplococci often acquire a local abscess at the point of inoculation.

Micrococcus lanceolatus under these conditions is an exquisitely pyogenic microorganism. The abscess which forms at the point of inoculation may be small or large. Usually the animal recovers.

There may be a combination of purulent formation with the preceding types of pneumococcus infection. Thus it is not uncommon to find small purulent foci mingled with the fibrinous exudation in the subcutaneous tissue. The first effect of subcutaneous inoculation may be an indurated spreading fibrinous exudation, and in course of time this may be resolved into one or more abscesses, with recovery of the animal.

An interesting but rare form of pneumococcus disease is that in which there are multiple suppurative processes in the body. These occur especially in the joints and in the serous cavities. In the Appendix, Case F, is related an example of purulent pleurisy and pericarditis, with localized subcutaneous abscess following subcutaneous inoculation, without pneumococci in the blood and organs, although the cocci were abundant in the pus. It is noteworthy that in animals as well as in man the pneumococcus is capable of producing not only fibrinous but also genuinely purulent multiple inflammations of the serous membranes without septicaemia.

Foà and Carbone[63] have observed that occasionally a rabbit which has been inoculated with pneumonic exudate taken from the dead body dies with symptoms of marasmus after 8 to 15 days, without any microorganisms in the blood and tissues. They infer that the diplococci were dead, leaving behind only the poison which they have produced. Other experimenters have had similar results. I have also met similar cases not only after inoculation with pneumonic exudate, but also after inoculation with the blood of rabbits dead of typical pneumococcus septicaemia. Such a case of peculiar interest is related in the Appendix, Case C, Exp. 5. In this instance a rabbit, the third in series, was inoculated subcutaneously with 0.8 c. c. of the heart's blood of a rabbit which had just died of typical pneumococcus septicaemia, 48 hours after inoculation, with many characteristics diplococci in the blood which developed pure in the cultures. This third rabbit died 15½ days after the inoculation without any living bacteria at the seat of inoculation, or in the blood and tissues, but with the most exquisite focal necroses in the liver and other evidences of toxaemia. As these lesions have not hitherto been described as the result of intoxication with the poison of *Diplococcus Pneumoniae,* I would call special attention to the protocol of this experiment. The explanation of the result in this case seems to me to be that with the pneumococci are introduced substances possessing both toxic properties and healing or immunizing properties. The peculiar result seems attributable to the mutual reaction of these different factors. This view is supported by the observation which has been made that rabbits may die in a similar way, without microorganisms in the blood and tissues, after simultaneous inoculation of virulent pneumococcus material and healing blood serum (Foà and Carbone). It is not very uncommon for mice to die in two to six days after inoculation with pneumococcus material without any bacteria being demonstrable in the blood and tissues after death.

The possibility of the recovery of rabbits after actual infection or intoxication has followed inoculation with the pneumococcus material cannot, I think, be denied, but we possess very little information on this point. There are occasionally late deaths without apparent cause one or two months or more after such inoculation. We pay in general too little attention in our experiments to these remote effects of bacterial inoculation. I have recently killed, three months after inoculation, a rabbit which for a week seemed to be very ill after inoculation with a pneumococcus culture which had killed a mouse with acute septicaemia in 36 hours. The rabbit did not develop an abscess and had apparently entirely recovered. I found the liver enlarged, very hard and pale, somewhat suggestive of waxy degeneration which was not present. This liver was the seat of extensive diffuse cirrhosis with so-called multiplication of the bile-ducts. There were no psorosperms or other parasites present. No microorganisms could be found. In view of the necrotic changes so commonly found in the livers of rabbits dead of pneumococcus infection, it does not seem improbable that the cirrhosis was dependent upon the inoculation from which the animal had evidently suffered three months previously. Banti[52] reports two cases of hepatic cirrhosis in guinea pigs resulting from chronic infection following peritoneal inoculation of one of his varieties of the pneumococcus.

It has already been mentioned that *Micrococcus lanceolatus* may be present in inflammatory exudates, including croupous pneumonia, as well as in cultures in a condition totally devoid of virulence, incapable of producing any demonstrable lesion or effect even in mice.

Having now passed in review the varied pathological effects which follow the ordinary subcutaneous or intravenous modes of inoculation, we turn to the efforts which have been made by special mode of inoculation to produce certain definite localizations of the morbid process.

In general it may be said that whether the inoculation be under the skin, into the circulation, into the serous cavities, into the cranial cavity, into the lungs or into the articular cavities, results similar to those already described are obtained, but some points of especial interest have been brought out by these diverse methods of inoculation.

The chief interest attaches to the efforts which have been made to reproduce in animals acute lobar pneumonia.

It is not very uncommon to find in pneumococcus infections of rabbits after subcutaneous or other methods of inoculation circumscribed areas of pulmonary hepatization. Intratracheal and intrapulmonary inoculations and inhalations of pneumococci have been resorted to for the purpose of producing with greater certainty pneumonia. The results obtained by different experimenters have varied considerably.

Emmerich[64] reported to the Seventh International Congress of Hygiene in London, in 1891, that Doenissen, working under his direction, had found that an hour's inhalation of sprayed bouillon cultures of pneumonia diplococci produced invariably in rabbits pneumonia and death in two to four days.

The injection of very virulent pneumonia cocci into the lungs or into the trachea of rabbits is usually followed, as might be expected, by acute septicaemia without especial localization in the lungs. It is evident that in order to produce pneumonia in this way we must either select pneumococci of weakened virulence so that they will not kill by quick septicaemia, or we must choose animals less susceptible than the rabbit and which respond to highly virulent pneumococci by spreading local inflammation rather than by acute septicaemia.

The early investigators, A. Fraenkel,[24] Weichselbaum[27] and Foà and Bordoni-Uffreduzzi[29] found that the intrapulmonary injection of pneumococcus cultures in rabbits is often followed by pleurisy, pericarditis and lobular or even lobar pneumonia, and these observations have been repeatedly confirmed. Monti[42] produced by intratracheal injection of cultures three to five days old in rabbits, "typical fibrinous pneumonia" of one lobe or of an entire lung, often complicated by pericarditis and sometimes almost without any general infection. The result, he says, depends on the mode of inoculation, the degree of attenuation of the organisms and the strength and development of the animal. Young animals responded by general infection sometimes when old ones acquired typical pneumonia. Lipari[65] claims that the susceptibility of rabbits and guinea pigs to pneumonia by intratracheal inoculation of attenuated pneumococcus material can be heightened by exposure to cold.

Gamaleia[56] claims to have produced in many instances typical fibrinous lobar pneumonia by the injection of extremely virulent pneumococcus cultures in large doses into the lungs of dogs and sheep.[5a] Endotracheal inoculations were without effect in these animals unless preceded by the injection of irritating substances like tartar emetic.

Tchistovitch[57] inoculated cultures of the pneumococcus into the trachea of 19 dogs. Of these three died of pneumonia, four acquired benign pneumonia (of these four, three were killed and one recovered), and the remaining twelve bore the inoculation without notable reaction, except temporary elevation of temperature.

It is not to be doubted from the observations mentioned, and many more might be collected in the literature of this subject, that under certain conditions *Micrococcus lanceolatus* is capable of causing acute lobular and even lobar pneumonia in animals. The statements of writers are often positive as to the identity of some of these experimental pneumonias with acute lobar pneumonia as it occurs in man. On this point, however, I think there is room for scepticism. Acute lobular pneumonia as it occurs in human beings is a very definite and well characterized affection both anatomically and clinically. So far as known, the domestic animals are not subject to a form of pneumonia etiologically and anatomically identical with croupous pneumonia of man. Bacteriologists are not always pathologists, and the bare statement that the pneumonia produced experimentally is in all respects identical with acute lobar pneumonia in human beings should be received with caution. But fortunately it is not necessary to insist upon the absolute identity of the experimental pneumonias caused by the pneumococcus with human acute lobar pneumonia. We possess conclusive experimental proof that *Diplococcus pneumoniae* is capable of producing spreading inflammatory exudates with all of the characteristics of the exudate in croupous pneumonia as it occurs in man. The evidence that this microrganism is the cause of acute lobar pneumonia in man rests upon many points which will be summarized later in this article and which seem to constitute incontrovertible proof.

[5a] These experiments of Gamaleia seem conclusive enough, but they are not described with sufficient detail to permit critical judgment, nor have they been satisfactorily confirmed by other investigators. Many inoculations of cultures of virulent pneumococci into the trachea and lungs of dogs have been made in my laboratory by Dr. Canfield and myself, but in no instance were we able to produce an inflammation of the lungs which we were willing to identify with acute lobar pneumonia as found in human beings. In the majority of our experiments there was no appreciable effect; in a few pleurisy and more or less extensive areas of pneumonia were produced. Our experience in this respect is analogous to that of Kruse and Pansini. The experiments cited of Monti and Lipari also await confirmation.

As *Micrococcus lanceolatus* is the cause of many cases of cerebrospinal meningitis (hence the name meningococcus), the attempt has been made to reproduce this lesion experimentally. A. Fraenkel[25] says that he has not been able to produce meningitis by inoculation, but the method of inoculation is not stated. Foà and Bordoni-Uffreduzzi,[25] by subdural inoculation of a meningococcus culture, produced in the rabbit "general infection with swelling of the spleen and meningitis endocranica totalis and hyperaemia of the spinal meninges." Similar results are reported by Netter,[45] Weichselbaum[55] and others. In these cases the exudation was very slight. Monti[43] produced by submeningeal inoculation in dogs of 24 hour pneumococcus cultures "typical cerebrospinal meningitis," while the corresponding experiments in rabbits gave less typical results.

Endocarditis is a very rare result of simple subcutaneous or intravenous inoculation, but it has been observed by Kruse and Pansini[38] and by Vanni.[59] It can be produced by the well known method adopted for causing infectious endocarditis, viz., by first injuring the valves by means of a sterilized probe passed from the carotid artery into the left ventricle and then injecting the cultures. In this way first Netter[60] and then Guarnieri[37] have succeeded in producing experimentally in rabbits by injection of pneumococcus cultures, vegetative and ulcerative endocarditis.

Gabbi[61] has produced experimentally in rabbits arthritis and periarthritis by direct injection into the joints of pneumococcus cultures, and also by subcutaneous injection following injury of the joint by traumatism or irritating substances.

Zaufal[62] has produced otitis media by inoculation of pneumococci into the tympanic cavity of animals.

It is evident from the foregoing review of the pathogenic effects produced by *Micrococcus lanceolatus* in animals, that we possess a rich mass of experimental data to aid us in explaining the multiform manifestations of infection by this microorganism in human beings.

The question as to how far we are justified in recognizing distinct varieties of *Diplococcus pneumoniae* will be answered differently by different bacteriologists, according to one's conception of the meaning in biological classification of the term variety. Those who insist that in order to justify the designation variety there should be a considerable degree of fixity and transmissibility of distinctive characters will find it difficult to establish well defined varieties of this organism. It seems to me, however, that we must give a somewhat looser meaning to the term variety in the classification of bacteria, and that we cannot conveniently dispense with this word in speaking of the different modifications of *Micrococcus lanceolatus*. These modifications are endowed, as we have seen, with very diverse pathogenic attributes.

Transmission of Infection from Mother to Foetus

In this connection a few words may be said concerning the transmission of the pneumococcus from the mother to the foetus in utero and concerning the presence of the organism in the secretion of the mammary gland.

It is well known that acute lobar pneumonia developing during pregnancy is likely to bring about miscarriage. The same is to be noticed in mice and rabbits inoculated with virulent pneumococci. Neither in human beings nor in animals does abortion necessarily attend pneumococcus infection.

The transmission of pneumococci from the mother to the foetus has been demonstrated both in human beings and in animals.

In the human cases reported as intrauterine infection (only those in which satisfactory bacteriological examination was made are here considered) by Netter,[63] Levy,[64] Birch-Hirschfeld[65] (first of his two cases) and Viti[66] the infants died two to five days after birth and, although the infection was probably intrauterine, the possibility of its occurrence during or after birth cannot be absolutely excluded. Foà and Bordoni-Uffreduzzi,[29] however, report finding pneumococci in the blood and liver of two human embryos of the fourth and of the sixth month born on the second and the third day of croupous pneumonia in the mother. Diplococci were present also in the uterine veins and the placenta. Birch-Hirschfeld's[65] second case is a particularly satisfactory one. On the fourth day of croupous pneumonia the mother gave birth to a seven months' still-born foetus with intact membranes. There were haemorrhages in the maternal and middle parts of the placenta. The only lesion noticed in the foetus was ecchymoses in the renal pelvis. Typical virulent pneumococci were found by culture in the liver, heart's blood and umbilical vein of the foetus, also in the placenta.

Actual pneumonia existed only in the infants who had lived some hours after birth, so that Netter concludes that it is necessary that the child should have breathed in order to acquire localization of the pneumococcus infection in the lung. As the still-born infants presented no characteristic lesion, only a bacteriological examination could have determined the existence of the infection.

It is probable that the transmission of pneumococci from mother to foetus is exceptional in the case of human beings. Levy did not find any organisms in a five months' foetus born when the mother was affected with croupous pneumonia.

There must of course have been pneumococci circulating in the blood of the mother in order to produce intrauterine infection, and in addition it is probable that some lesion of the placenta, such as haemorrhages, is, if not absolutely necessary, at least an important predisposing factor.

In pregnant rabbits with pneumococcus septicaemia infection of the foetus in utero is far from constant, but it has been repeatedly observed.

Ortmann[67] has seen metritis and endometritis in a pregnant guinea pig infected with *Diplococcus lanceolatus.*

Foà and Bordoni-Uffreduzzi[29] were the first to note the presence of pneumococci in the milk of pregnant rabbits inoculated with the virulent organism. They furthermore obtained diplococcus septicaemia in young rabbits which sucked the breast of the mother infected with the lanceolate coccus. The same authors have found pneumococci in the milk of women affected with croupous pneumonia, and this observation has been confirmed by Bozzolo.[68] We have no information as to the frequency of this occurrence.

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THE ETIOLOGY OF DIPHTHERIA[1]

In 1884 Löffler, in an article published in the second volume of the "Mittheilungen aus dem Kaiserlichen Gesundheitsamte," concluded from the bacteriological examination of a large number of cases of diphtheria that a species of bacillus found by him frequently in diphtheritic membranes and carefully studied in its morphology and biology, and identified with the one described the previous year by Klebs, is the only one present which can be supposed to be of pathogenetic importance. He expressed himself, however, for several reasons with commendable reserve as to the etiological significance of this bacillus. Subsequent researches of a large number of investigators have removed one by one the doubts which Löffler had expressed until at the present time nearly all bacteriologists are convinced that the Klebs-Löffler bacillus is the specific cause of primary diphtheria. As the results reached by these various investigators have been summarized not long since by Löffler,[2] it is not necessary to describe them here in detail. It will suffice to mention the names of Babes, d'Espine, v. Hoffmann, Ortmann, Roux and Yersin, Kolisko and Paltauf, Zarniko, Sörensen, Escherich, Klein. Since the publication of Löffler's critical review in 1890, the most important contributions have been the investigations of the toxic albumens produced in cultures of the diphtheritic bacillus by Fränkel and Brieger, and the experimental production of immunity against inoculation with the diphtheritic bacilli or their poisonous products by Fränkel and by Behring.[3] By these investigations it has been determined that the Klebs-Löffler bacillus is present in all cases of primary diphtheria in the diphtheritic deposits, that it does not invade the blood or organs or even the mucous membrane affected, that in susceptible animals the disease can be reproduced in all its features, even to the production of paralysis, that the constitutional symptoms, the paralysis and the changes peculiar to diphtheria in internal organs are caused by a toxic albuminous substance or substances which have been separated in a condition approaching purity and which are produced by the local action of the bacilli in inoculated animals as well as in cultures, and that by various methods a greater or less degree of immunity can be artificially conferred upon susceptible animals and that under certain conditions susceptible animals can be cured after inoculation with virulent cultures.

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[1] William H. Welch and Alexander C. Abbott. The results of the investigations reported in this paper were communicated to the Johns Hopkins Hospital Medical Society, Baltimore, June 2, 1890, and January 9, 1891.

Johns Hopkins Hosp. Bull., Balt., 1891, II, 25-31.

[2] Löffler: Der gegenwärtige Stand der Frage nach der Entstehung der Diphtherie. Deutsche Med. Wochenschrift, 1890, Nos. 5 and 6.

[3] Fränkel and Brieger: Berliner klin. Wochenschrift, 1890, Nos. 11 and 12. Fränkel: Berliner klin. Wochenschrift, 1890, No. 49. Behring: Deutsche med. Wochenschrift, 1890, No. 50.

In view of these convincing results it might be thought unnecessary to publish any further observations upon the bacteriological examination of cases of diphtheria; but Löffler, in the critical review mentioned, has called attention to the apparently discordant observations which have been made in this country by Prudden. In 24 cases of diphtheria in children examined by Prudden[4] he failed to find in a single instance, either by microscopical examination of the membranes or by cultures, the Klebs-Löffler bacillus. Recognizing the thorough competence of Prudden as a bacteriologist, Löffler says, "I do not believe that in North America a form of diphtheria prevails different from that with us. With us the bacilli are found regularly by every investigator, there they are constantly missed. Further investigations must and will clear up this contradiction." And he concludes his article with the sentence, "A simple 'Liquet' cannot be affirmed until investigations inconsistent with this, such as those of Prudden in North America, have been shown to be erroneous."

We consider that the excellence of his methods, his well known thoroughness and accuracy and his well deserved reputation as a bacteriologist, give to the results published by Prudden the greatest weight. Nor do we regard it necessary to demonstrate that his observations are erroneous as suggested by Löffler. In the estimation of his results the following points mentioned in his article ought to be considered. All but two of the twenty-four cases occurred in children's asylums or hospitals, 16 being in a large foundling asylum in which measles and scarlatina were prevalent. In 7 cases the diphtheria complicated or was preceded by measles, in 3 by scarlatina, in 1 by whooping cough, in 4 by phlegmonous or erysipelatous inflammation, and in only 9 was the diphtheria apparently uncomplicated by notable previous disease; 4 cases were in children under one year of age, one of these being 6 days and another 7 days old. Of the apparently uncomplicated cases it is not clear from the article how many developed in the hospital under the same epidemic influences as the majority, but that some did is expressly stated.

It seems to us, therefore, clear that the cases examined by Dr. Prudden, taken as a series, should not be regarded as cases of primary diphtheria, but rather as diphtheria, secondary to scarlatina, measles, erysipelas, or as developing in a situation where these diseases prevailed. While we do not possess all of the information desirable as to the etiology of the pseudomembranous anginas secondary to various infectious diseases, and while we know that some of these are etiologically identical with primary diphtheria, it is certain that many of these cases should be separated from primary diphtheria. It has been shown by Wurtz and Bourges,[5] as well as by others, that the pseudomembranous angina, occurring in the early stages of scarlatina, is accompanied regularly by a streptococcus probably identical with that found by Prudden, and not by the Klebs-Löffler bacillus. While it seems remarkable that in not a single instance, even of the apparently primary cases of diphtheria, did Prudden find the Löffler bacillus, we consider, for the reasons stated, that his observations need not be interpreted as contradictory to the conclusions of others who have studied cases of primary diphtheria. Nevertheless, it is evidently desirable that further bacteriological examination of cases of diphtheria occurring in this country should be made—the more so in view of the statements of Löffler already quoted.

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[4] Prudden: Am. Jour. of the Med. Sciences, April and May, 1889.

In May, 1890, we therefore began the bacteriological examination of the membranous exudations in cases of diphtheria occurring in Baltimore. At the time of our examinations diphtheria was not prevailing as an epidemic, and we were unable to secure a large number of cases. In consequence, however, of the uniformity of the results obtained, and of their correspondence with those of European investigators in hundreds of cases, it did not seem necessary to examine a very large number in order to establish the etiological identity of our diphtheria with that in other countries. We have, however, waited before publishing our results in order to obtain more cases, but as we have not been fortunate in this respect, we have decided not to wait longer for additional cases.

In all of the cases of diphtheria examined by us the clinical diagnosis was positive, and all were primary diphtheria developing in healthy children. All of the cases were in private practice and occurred in different parts of the city. For the opportunity of examining the cases we are indebted to Drs. Jay, Cordell, Lockwood, Booker, McCormick and Neff. The examinations were all made with bits of membrane removed during life. At first we removed these bits from the tonsils, or soft palate or pharynx, by means of sterilized forceps, but we afterward found it sufficient, as a rule, to touch or scrape or slightly bore into diphtheritic exudations in these situations with a sterilized platinum wire loop which had been flattened. Small particles of diphtheritic membrane thus removed, were transferred to a sterilized test tube, and carried to the laboratory where they were at once examined.

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[5] Wurtz and Bourges: Archives de Médecine Expérimentale, T. II, p. 341, 1890.

In each case cover-glass preparations were made from the membranous particles, and then, usually after washing in sterilized salt solution, a series of smear cultures was made in five or six tubes of slanting glycerine agar, or of Löffler's blood serum and bouillon mixture, and a series of three or four plate cultures with glycerine agar (Petri's dishes being used) was prepared. When the material sufficed we likewise often made similar cultures in nutrient gelatine or plain nutrient agar. Only in the last two cases which we examined did we make use of the blood serum and sugar bouillon mixture recommended by Löffler, upon which we found the diphtheritic bacillus to grow much more rapidly and luxuriantly than upon any of the other media used, but as at first we did not have this medium prepared, and as we nevertheless had no difficulty in obtaining positive results with the other media, particularly the glycerine agar, we continued to use the latter in most of our work. There are, however, distinct advantages in the use of the blood serum bouillon medium, upon which the colonies of the Klebs-Löffler bacillus at a temperature of 35° C. are readily recognized in 18 to 24 hours. The study of the colonies we found a decided help in the isolation of the diphtheritic bacillus, and their characteristic features are well marked in glycerine agar plate cultures, which, therefore, may be recommended as a most useful medium for isolating the organism. After separating the bacillus in pure culture, its virulence was tested in each case by inoculation of guinea pigs. We had for comparison a pure culture of the Löffler bacillus, obtained from the Hygienic Institute in Berlin, for which we are indebted to Dr. von Esmarch. The identification of the bacillus, in our cases, was based therefore upon the study of its morphology, its behavior in various culture media, and its effects when inoculated into guinea pigs and other animals. In all respects we found it identical with the Löffler bacillus in the culture obtained from Berlin.

The following is a brief summary of the cases examined:

Case 1.—Negro girl, 7 years old, has been sick two days. A thick whitish membrane covers tonsils, pharynx and anterior surface of uvula and soft palate. The child recovered. A piece of membrane 2 mm. thick and 5 mm. long was removed with forceps. Microscopical examination showed the usual structure of a diphtheritic membrane. Cover-glass preparations showed, singly and in clumps, bacilli averaging about the length of tubercle bacilli but thicker, often with one, sometimes with both ends swollen, frequently in one or both ends a small round body staining more deeply with methylene blue than the rest of the bacillus. These bacilli sometimes appeared slightly curved or broken into irregular segments. In addition, in considerable number, were found cocci single, in pairs and occasionally in chains, and a few long, slender bacilli were present.

Glycerine agar plate cultures showed in No. 1 diffuse cloudiness from abundant growth, in No. 2 very numerous scattered small colonies, and in Nos. 3 and 4 well distributed colonies. With the exception of 4 large white colonies of a coarse coccus in No. 1, only colonies of a streptococcus and of the Löffler bacillus were recognized, these two being in about equal number. The surface colonies of the Löffler bacillus were readily recognized in cultures two or three days old by their whitish centre, grayish translucent periphery, thin, dry appearance, and, under the microscope, the light brownish centre, the coarsely granular periphery (the granules appearing rather more as short lines than as granules) and slightly wavy or irregular contours.

A guinea pig inoculated subcutaneously with 0.7 c. c. of a suspension in sterilized NaCl solution from a pure culture of the diphtheritic bacillus from this case, died in 60-70 hours with extensive local oedema, slight swelling of spleen, irregularly mottled kidneys, swollen and reddened suprarenal capsules, and moderate increase of pericardial and pleural fluids. The diphtheritic bacilli were obtained in pure culture from the oedematous fluid, but tubes inoculated from the lymph glands, blood, spleen, liver and kidney remained sterile.

As the results and methods in subsequent cases are similar, they can be described more briefly.

Case 2.—Negro woman, 18 years old, living in a family in which one child had recently died of diphtheria and four others are at present either sick or recovering from the disease, is said to have been sick about ten days, has a thick, rather tough, coherent, whitish membrane on uvula and soft palate. Patient died.

Cover-glass specimens showed, with the exception of very few streptococci and still fewer large cocci in clumps, only the Löffler bacillus, and this in large number, so that the specimens look almost like pure cultures.

Glycerine agar plates showed only in No. 1 very few streptococcus colonies, and large white colonies of a coarse coccus. The rest were colonies of the diphtheritic bacillus which appeared in pure culture in the numerous colonies of No. 2 and the scattered colonies of No. 3. Guinea pig died with characteristic lesions in 48 hours.

Case 3.—Mulatto boy, 4 years old. Three children in same family had been attacked with diphtheria and one had died. Grayish white membrane on both tonsils and pharynx. Patient died.

Cover-glass specimens showed the Löffler bacillus as the predominant organism in large number. No streptococci found.

Cultures in glycerine agar were almost pure of the diphtheritic bacillus, the colonies being very abundant. No streptococcus colonies could be found. Guinea pig died in 36 hours with characteristic lesions.

Case 4.—Mulatto girl, 3 years old. Tonsils and posterior wall of pharynx covered with yellowish gray diphtheritic membrane. Patient recovered.

Cover-glass preparations showed a large number of Löffler bacilli, mostly with swollen ends, sometimes of fusiform shape, also many short oval or slightly pointed bacilli and a few cocci, but none were found in chains.

Cultures in glycerine agar presented, in about equal number, colonies of the Löffler bacillus and of a short oval bacillus. A few colonies of a coccus, occurring in short chains of five or six cocci, were found. Guinea pig died with the characteristic lesions in 50 to 60 hours.

Case 5.—Mulatto girl, 7 years old, in same family as preceding case. Firmly adherent tenacious gray membrane on left tonsil. Patient recovered.

Cover-glass specimen showed a large number of characteristic Löffler bacilli with their so-called involution forms, also many short oval bacilli, sometimes in clumps, a few coarse long bacilli, and a few large cocci. No streptococci.

The cultures and inoculation of guinea pig gave the same results as in the preceding case.

Case 6.—Mulatto boy, 6 years old, in same family as case 3. Thin gray adherent membrane covered both tonsils and was present also on pharynx. Patient died.

Cover-glass preparation showed as the predominant organism the Löffler bacillus in the form often of slightly curved club shaped bacilli, frequently in clumps. In addition, short oval bacilli and cocci were present in considerable number.

The glycerine agar plate cultures showed a large number of colonies of the Löffler bacillus, and a nearly equal number of colonies of *Staphylococcus pyogenes aureus*. A few streptococcus colonies were found and a large number of colonies of a short oval bacillus, occurring frequently in long chains. A guinea pig died in 36 to 48 hours with the usual lesions.

Case 7.—Girl, 7 years old. Sick for 3 days. Both tonsils covered with grayish yellow diphtheritic membrane. Patient recovered.

Cover-glass preparations showed Löffler bacilli, but not in so large number as various other organisms of different shapes including long thin rods and threads, short oval bacilli, large cocci, sarcinae, etc.

Glycerine agar and blood serum cultures showed a moderate number of characteristic colonies, on the latter medium impure, of Löffler's bacilli and a larger number of colonies of various kinds of bacteria, chiefly streptococci, short oval bacilli and yeast fungi. A guinea pig died in about 72 hours with characteristic lesions.

Case 8.—Boy, 3 years old. Has been sick 4 days with fever and symptoms of croup. Examination of throat difficult on account of struggles of patient, but certainly no pseudomembrane is visible on the tonsils or pharynx, but the attending physician thinks that he has detected it in the larynx. The breathing of the child indicates laryngeal obstruction and the cough is croupal in character. Notwithstanding repeated observations no false membrane could be detected at any time in pharynx or on tonsils or soft palate. Some mucopus was scraped from the back of the pharynx and used for cultures. Patient recovered, improvement beginning in 5 days after onset of symptoms.

Blood serum cultures showed a few large white colonies of a coarse coccus and a very large number of colonies of the Löffler bacillus, the latter representing almost a pure culture. A guinea pig died after inoculation on the third day with characteristic lesions.

The preceding eight cases are all of the cases of diphtheria which we have had an opportunity of examining by culture methods. All were cases of primary diphtheria in which, with the possible exception of case 8, no doubt existed to the clinical diagnosis of diphtheria. In case 8, the membranous deposit was limited to the larynx and trachea, the case being therefore one of primary laryngotracheal diphtheria, or so-called membranous croup, but in this case we obtained a nearly pure culture of the Löffler bacillus from the pharynx. The cases developed in patients living in widely separate parts of the city. In every case we found the Löffler bacillus and in most cases both on cover-slip preparations and in cultures this organism was the predominant one present, existing in the bits of membrane examined in nearly pure culture.

We have also made bacteriological examinations in a considerable number of cases which have served as a control for the cases of diphtheria. A few of these control cases have been children without any affection of the throat, most of them have been cases of simple inflammation of the tonsils or pharynx in which no suspicion of diphtheria existed, four have been cases of somewhat suspicious whitish exudation upon the tonsils, so-called follicular tonsilitis, in which at first the possibility of the existence of diphtheria has been entertained by the attending physician, but the subsequent history of the case has shown that it was not diphtheria. In one case we have made a bacteriological examination of the pharyngeal secretion three weeks after the diphtheritic membrane had disappeared. In none of these cases have we found the Löffler bacillus, nor any bacillus which an experienced bacteriologist would be likely to confound with the Löffler bacillus. We have not met with the so-called pseudodiphtheritic bacillus, but in view of the limited number and special character of the cases which we have studied, we are not prepared to draw any conclusions as to the prevalence of the latter species.

The results of the bacteriological examination in the cases where at first there was some suspicion of diphtheria which the subsequent course showed to be unfounded, have convinced us as to the great practical value for diagnostic purposes of a bacteriological examination by cover-glass specimens and by cultures in this large group of cases often so puzzling to the practitioner.

The following case is an illustration:

A boy 8 years old was brought to the dispensary of the Johns Hopkins Hospital. Nearly the whole of the right tonsil was covered with a soft yellowish white membranous mass which could be easily removed with the forceps. The boy had fever but did not appear very sick. Cover-slip preparations showed a variety of microorganisms, the most conspicuous ones being cocci both in groups and in chains, rather short thick rods, and spirochaete. The cultures showed as the predominant organism colonies of streptococci and of *Staphylococcus pyogenes aureus*. Notwithstanding extended search not a single colony of the Löffler bacillus was found. In three days the inflammation had disappeared and the boy was well. None of several children living in the same apartments developed any soreness of the throat. We have examined with similar results three other analogous cases.

We have had the opportunity of examining microscopically two large pieces of diphtheritic membrane which had been expelled by patients living at a distance and which hardened in alcohol were sent to the laboratory. Sections of these membranes after staining by Gram's method showed in clumps and singly the characteristic Löffler bacilli which could be readily identified by their peculiar shapes and staining.

The only species of bacteria which we have found constantly in the cases of diphtheria has been the Löffler bacillus. Two other species have been present in many cases, viz.: the well-known streptococcus, which grows in much smaller colonies and less rapidly than the Löffler bacillus and a short oval, often slightly pointed bacillus, growing in long chains running parallel to each other. There are often marked irregularities in shape and especially in size of this bacillus, even of individuals in the same chain. The colonies of this organism are grayish white, moist, larger than those of the streptococcus but smaller than those of the Löffler bacillus. The colonies of this short bacterium bear more resemblance to those of the Löffler bacillus than any others which we have met with, although colonies of other organisms bear a greater similarity in rapidity of growth and in size, notably those of a large coccus, but they are readily distinguished by a more opaque white appearance.

We have not utilized any of the material from the cases of diphtheria which we examined by culture methods, in order to study the distribution of the bacteria in the membranes, as the material rarely sufficed for hardening and making sections. We have, however, studied sections prepared by Dr. Councilman from a large number of cases of diphtheria. These were of cases of diphtheria dying in Frankfurt, Germany, the sections being made under Prof. Weigert's direction, and stained by his new modification of the fibrin stain, the details of which have not yet been published. Upon these sections both the fibrin and the Löffler bacilli are brilliantly stained, and the diphtheritic foci described by Oertel in the mucous membrane and in lymphatic glands and elsewhere are well shown. The Löffler bacilli, with their irregular, often bent, shapes, segmented appearances and deeply stained granules, are clearly to be seen. The usual arrangement is as follows: Most superficially is a deeply stained mass containing various kinds of bacteria, among which the Löffler bacilli are to be recognized; beneath this comes a feebly stained layer, rich in cells, but containing little or no fibrin; in this layer the Löffler bacilli, both singly and in clumps, are much more readily made out. In the deeper parts of this pale layer, the Löffler bacilli are often the only bacteria present, and especially just above the deeply stained fibrin layer, characteristic clumps of these bacilli are present. Streptococci may, however, also be present in this layer. The layer resting upon the mucous membrane is rich in fibrin and therefore stains deeply with the Weigert stain. In this fibrin layer, except in its most superficial parts, no Löffler bacilli are to be seen. This arrangement of the bacilli corresponds with the description given by Klebs and by Löffler. We have never found the bacilli in the mucous membrane affected nor in sections from internal organs, not even in the diphtheritic foci presenting nuclear fragmentation and hyaline masses and fibrin. The Löffler bacilli, therefore, not only do not invade the affected mucous membrane; they do not usually even extend throughout the entire thickness of the diphtheritic pseudomembrane. On the other hand, various complications of diphtheria may be due to the secondary invasion of other organisms, notably streptococci, which are occasionally found in the mucous membrane, lymphatic glands and elsewhere.

Our studies of the morphological and biological properties of the Klebs-Löffler bacillus are, in the main, simply confirmatory of the results of other investigators, and we shall therefore give only a brief description of this part of our work, emphasizing only certain points which are not clearly brought out in the publications of previous observers.

The bacillus is nonmotile. It varies greatly in size and shape, averaging 2.5 to 3 μ in length and 0.5 to 0.8 μ in thickness. Its morphological characters are so peculiar as to render its identification on cover-slip preparations and on sections from diphtheritic membranes in most cases an easy matter. Sometimes, appearing as a regular straight or slightly bent rod, with rounded ends, it is especially characteristic to find irregular and often bizarre forms, such as rods with one or both ends swollen, and very frequently rods broken at irregular intervals into short sharply marked segments, either round, oval or with straight sides. Some forms stain uniformly, others in various irregular ways, the most common being the appearance of deeply stained granules in a lightly stained bacillus.

Most writers are silent upon the question as to whether the Löffler bacillus stains by Gram's method or not. It is stated by Beck[6] that in sections it cannot be stained either by Gram's or by Weigert's fibrin method. This is contrary to our experience. We find that both in sections and in cover-glass specimens from blood-serum cultures, the bacilli stain well by Gram's method. Only by prolonged decolorization of cover-glass preparations with alcohol and oil of cloves do most of the bacilli lose their color. We find both Gram's and Weigert's fibrin method admirably adapted for the staining of sections of diphtheritic membranes in order to demonstrate the Löffler bacilli. Nothing can surpass in brilliancy and sharp differentiation, sections stained doubly by the modified Weigert's fibrin stain and picrocarmine.

The Löffler bacillus can be cultivated readily upon all of the ordinary culture media, viz.: alkaline bouillon, nutrient gelatine, nutrient agar, glycerine agar, blood serum and potato. The medium upon which it grows most rapidly and luxuriantly, and which is best adapted for determining its presence in diphtheritic exudations, is the mixture of blood serum and bouillon as recommended by Löffler (three parts blood serum and one part bouillon, containing 1 per cent peptone, 1 per cent grape sugar and 0.5 per cent chloride of sodium; the mixture, of course, to be solidified in the usual way for blood serum). For blood serum can be substituted serous transudations, but these have not seemed to us quite equal to the blood serum. On the blood serum mixture the colonies of the Löffler bacillus grow so much more rapidly than other organisms usually present in the secretions and exudations in the throat that at the end of twenty-four hours they are often the only colonies which attract attention, and if other colonies of similar size are present they are generally of quite different aspect. A blood serum tube, studded over with coalescent or scattered colonies of the Löffler bacillus, is so characteristic in appearance that one can anticipate with tolerable certainty the result of the microscopical examination. By this method of cultivation a diagnosis can usually be made on the day following the inoculation of the tube. The colonies are large, round, elevated, grayish white, with a centre more opaque than the slightly irregular periphery. The surface of the colony is at first moist, but after day or two rather dry in appearance.

The appearances of the young colonies in glycerine agar and plain nutrient agar are well depicted by Flügge.[7] We have found the bacillus to grow well in agar, as a rule, but often the first inoculations of a plain nutrient agar tube have given comparatively feeble growths while subsequent inoculations have afforded more abundant growths. Nutrient gelatine, of a feebly alkaline reaction, we have found an excellent medium at a temperature of 22° C., and we have repeatedly obtained pure colonies of the Löffler bacillus on this medium from diphtheritic deposits. Like Fränkel and Brieger,[8] we have sometimes observed a diffusely cloudy growth of the Löffler bacillus in alkaline bouillon, but, as a rule, the growth has been, as Löffler described it, in the form of small whitish specks at the bottom and along the sides of the tube, leaving the fluid clear. In bouillon cultures, standing a long time in the thermostat at 35° C., sometimes a soft whitish membrane forms over a part of the surface.[9] The reaction becomes at first acid and subsequently again alkaline, changes which can be well observed in cultivations in bouillon to which a little rosolic acid has been added.

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[6] Beck: Zeitschrift für Hygiene, Bd. VIII, p. 436.

[7] Flügge: Die Mikroorganismen, p. 229. Leipzig, 1886.

Löffler and following him most writers state that the diphtheritic bacillus does not grow on potato unless it is rendered alkaline. Zarniko,[10] however, says that after 8 to 10 days, at a temperature of 35° C., a growth of extremely distorted rods can be recognized as a delicate whitish deposit where the potato has lost its glistening appearance. Our experience has been that *Bacillus diphtheriae* grows on ordinary steamed potato without any preliminary treatment, but that the growth is usually entirely invisible or is indicated by a dry thin glaze after several days. Doubtless the invisible character of the growth has led most observers into the error of supposing that no growth existed, whereas the microscopical examination reveals a tolerably abundant growth, which on the first potato is often feebler than on succeeding ones. Irregular forms are particularly numerous in potato cultures, and in general the rods are thicker than on other media. In 24 hours, at a temperature of 35° C., microscopical examination shows distinct growth. We have cultivated the bacillus for many generations on potato.

We have tested the thermal death point upon bouillon cultures and suspensions in sterilized salt solution in capillary glass tubes and have found that the bacillus is killed at a temperature of 58° C. in ten minutes. It does not form spores.

We have used guinea pigs, rabbits and kittens for inoculation experiments with pure cultures.

Most of our inoculations have been made upon guinea pigs by subcutaneous injections of 0.1 to 0.7 c. c. bouillon cultures or dilute suspensions in sterilized salt solution of the Löffler bacillus or by inserting a platinum needle carrying a small quantity of the culture. The duration of life in these animals has varied from 24 hours to four to five days. The usual changes have been an extensive local oedema with more or less hyperaemia and ecchymosis at the site of inoculation, frequently swollen and reddened lymphatic glands, increased serous fluid in the peritoneum, pleura and pericardium, enlarged and haemorrhagic suprarenal capsules, occasionally slightly swollen spleen, sometimes fatty degenerations in the liver, kidney and myocardium. There are variations as to the extent and presence of these lesions in different cases which need not be here described. We have always found the Löffler bacilli at the seat of inoculation, most abundant in a grayish white fibrinopurulent exudate present at the point of inoculation and becoming fewer at a distance from this, so that the more remote parts of the oedematous fluid do not contain any bacilli. We find the bacilli not only free but contained in large number in leucocytes, some of which have fragmented nuclei or have lost their nuclei. The bacilli within the leucocytes as well as some outside sometimes stain very faintly and irregularly and may appear disintegrated and dead. We have never failed to obtain the bacilli in cultures from the point of inoculation and think it likely that the negative results of some observers in demonstrating the bacilli are due to making their examinations with the oedematous fluid at a place too remote from the point of inoculation. In all cases culture tubes inoculated with the blood, spleen, liver, kidney, suprarenal capsules, lymphatic glands and serous transudates, have yielded negative results.

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[8] Fränkel and Brieger: Berliner klin. Wochenschrift, 1890, p. 243.

[9] Noticed also by Roux and Yersin, Annales de l'Institut Pasteur, 1889, p. 274.

[10] Zarniko, Centralblatt f. Bakteriologie, Bd. VI, p. 180.

Rabbits are less susceptible, but they have usually died in the course of 5 to 20 days after inoculation of even small quantities. They develop as a rule paralysis affecting the posterior extremities, and we have also observed a peculiar tremulous motion and irregular respiration.

An affection may be reproduced by inoculation of certain animals in all respect identical with the disease diphtheria as it exists in man. The following case selected from several similar ones is an example:

A half-grown kitten is inoculated into the trachea with one platinum loop from a pure culture of the Löffler bacillus on glycerine agar, 11 days old, derived from Case 4. For the inoculation a small median incision was made over the trachea in which a hole just large enough to admit the platinum loop was made. The culture was rubbed over the mucosa of the trachea for an extent about 3 cm. in length, and in this process sufficient force was used to abrade the mucous membrane. On the day following the inoculation no special alteration in the animal was observed, but on the morning of the second day it was found very weak. In the course of this day it became so weak as to lie completely motionless, apparently unconscious with very feeble shallow respiration. Several times it was thought to be dead but on careful examination proved still to be breathing feebly. It was found dead on the morning of the 3rd day. At the autopsy the wound was found gaping and covered with a grayish, adherent, necrotic, distinctly diphtheritic layer. For a considerable distance around the wound the subcutaneous tissues were very oedematous, the oedema extending from the lower jaw down over the sternum and to the sides of the neck and along the anterior extremities. The lymphatic glands at the angle of the jaw were markedly swollen and reddened. The mucous membrane of the trachea beginning at the larynx and extending down for 6 cm. was covered with a tolerably firm, grayish white, loosely attached pseudomembrane in all respects identical with the croupous membranes observed in the same situation in cases of human diphtheria. No other noticeable lesion was found in the animal unless it be a greater degree of fatty metamorphosis of the renal epithelium than is normal in kittens. Both in the pseudomembrane and in the oedematous fluid adjacent to the wound many *Bacilli diphtheriae,* occurring often in clumps, were recognized on cover-slip preparations and this species grew in a large number of colonies on plate cultures. It is interesting to note that the faithful attendants of the Löffler bacillus, streptococci, were present also in the same situations.

The mere production of a pseudomembranous inflammation cannot be claimed as establishing by itself alone the etiological rôle of *Bacillus diphtheriae* as such an inflammation as is well known can be produced experimentally in a variety of ways. Greater significance attaches to the demonstration of certain peculiar alterations no less characteristic of the specific action of the diphtheritic virus. As in human diphtheria so in all susceptible animals inoculated with the Löffler bacillus, there is only a local development of the bacilli at the point of infection, and it has been demonstrated especially by Roux and Yersin, and by Brieger and Fränkel[11] that extraordinarily poisonous products formed by the bacilli are absorbed from the site of local development of the bacilli and give rise to the constitutional symptoms and the lesions of internal organs. The separation of these products in a condition approaching purity in the form of one or more toxic albuminous substances and the production by injecting them of paralyses and of other symptoms and lesions (save the pseudomembrane) of diphtheria are unquestionably brilliant achievements, and have added greatly to the chain of proof leading to the recognition of the Löffler bacillus as the specific cause of diphtheria. Our studies so far as they have been directed to this part of the subject are confirmatory of the results reported by Roux and Yersin and by Brieger and Fränkel.

We have directed our attention with especial interest to the question as to whether the interesting and important discoveries of Oertel,[12] relating to the special action of the diphtheritic virus upon cells and the appearance of peculiar foci of nuclear fragmentation and cell death with other changes in the lymphatic glands, and the spleen could be confirmed by experiments upon animals. Babes[13] has also communicated some observations upon this point. Dr. Flexner is at present engaged in the Pathological Laboratory in the examination of this question, and the results will be reported subsequently. Here we will only mention that, in a number of instances, we have been able to show not only extensive cell death characterized by an extreme degree of fragmentation of nuclei at the site of inoculation, but also similar changes in the spleen, lymphatic glands and elsewhere. These peculiar alterations, as Oertel has shown, in their distribution, are characteristic of human diphtheria, and the demonstration of similar changes in animals, inoculated with the Löffler bacillus, is no small additional proof that diphtheria is caused by this microorganism.

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[11] Roux and Yersin: Annales de l'Institut Pasteur, 1888, 1889, 1890. Brieger and Fränkel: Berliner Klinische Wochenschrift, 1890.

It would seem, therefore, that every postulate necessary to prove that the specific cause of primary diphtheria is the Klebs-Löffler bacillus has been fulfilled, and that this bacillus may be designated *Bacillus diphtheriae.* This bacillus is present in every case of primary diphtheria, in such number and situation as to explain the local manifestations of the disease, it can be readily isolated in pure culture, and a disease identical in all respects with human diphtheria can be produced experimentally by the inoculation of pure cultures. The investigation of the toxic albumens, produced by this remarkable bacillus, has cleared up many obscure points.

The only points which can be brought forward as tending to cast any doubt upon the recognition of this bacillus, as the specific cause of diphtheria, are the very exceptional observation of an apparently identical bacillus in the throat of healthy children and the more frequent observation of a bacillus, usually called the pseudodiphtheritic bacillus which differs from true *Bacillus diphtheriae* chiefly or only by the absence of pathogenic properties when inoculated into animals.

The finding in healthy individuals of a bacillus apparently identical with *Bacillus diphtheriae* is such a rare occurrence that Löffler, who made the first observation of this nature, has not been able to repeat it during several years of search. When found it has been present in such small number as to present a marked contrast to the results in cases of diphtheria. The exceptional occurrence in healthy persons of *Bacillus diphtheriae* in such small number as to indicate that it does not find suitable conditions for its multiplication and injurious action upon the tissues, seems to us in no way opposed to the acceptance of this bacillus as the specific cause of diphtheria. Experiments upon susceptible animals show that to produce in them a pseudomembranous inflammation of a mucous membrane by inoculation with the diphtheritic bacillus, it does not suffice simply to apply the bacilli to the surface of the mucous membrane but that an actual injury must be done to the membrane. While it is by no means proven that an injury to or a morbid condition of the mucous membrane is necessary for the infection of human beings with the diphtheritic virus, nevertheless such conditions are recognized as important predisposing causes, and clinical observations point to differences in susceptibility to infection with this virus, so that there is no reason why in some healthy persons *Bacillus diphtheriae* might not be present in the mouth without doing any damage.

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[12] Oertel: Die Pathogenese der epidemischen Diptherie nach ihrer histologischen Begründung, Leipzig, 1888.

[13] Babes: Centralblatt f. Bakteriologie, Bd. VIII, p. 741.

The status of the so-called pseudodiphtheritic bacillus cannot be considered as settled. Whereas Löffler finds such morphological and biological differences between it and true *Bacillus diphtheriae* as to lead him to regard it as unquestionably a different species, Roux and Yersin consider that it is simply a modification of *Bacillus diphtheriae,* from which it differs only by absence of virulence. They claim that the pseudodiphtheritic bacillus is identical in its morphology and its behavior on culture media with the diphtheritic bacillus, and, calling attention to the marked variations in virulence of the diphtheritic bacilli found not only in different cases but in the same cases of diphtheria, they assert that every transition exists between the most virulent diphtheritic bacillus and the nonvirulent pseudodiphtheritic bacillus. Although their observations and experiments have not proven their belief, they bring forward strong arguments in support of the view that *Bacillus diphtheriae* under certain circumstances may be converted into a nonvirulent variety and conversely the nonvirulent bacillus into the virulent form. The pseudodiphtheritic bacillus appears to be not particularly uncommon in the mouth and throat of healthy children or of children not affected with diphtheria, but when present it does not occur in anything approaching the number of diphtheritic bacilli ordinarily found in cases of diphtheria, so that a diagnostic error is not likely to result even in cases in which it exists. The inoculation of animals will settle the question. Whatever may prove to be the nature of the pseudodiphtheritic bacillus, it does not seem to us that the observation of a nonvirulent bacillus resembling or even identical morphologically and in modes of growth on culture media with *Bacillus diphtheriae* can be justly urged as an argument opposed to the recognition of the latter organism as the specific cause of diphtheria, and indeed certain facts in the development of diphtheria may possibly find their best explanation in the assumption that *Bacillus diphtheriae* may exist in a nonvirulent state, and under certain conditions which we do not now understand may acquire virulence.

There are few infectious diseases upon which greater light has been shed by the discovery and study of their specific microorganisms than has been the case with diphtheria.

We are now in possession of a positive means of diagnosis, and while it may be questioned whether practitioners of medicine in general are likely to be in a position to make use of this means, the method is not difficult and can be readily applied. We have already learned that there are pseudomembranous anginas which must be separated etiologically from true diphtheria, and also that diphtheria may exist in extremely mild forms even without visible pseudomembranous deposits. We are now in a position to clear up a hitherto confusing and much disputed chapter in etiology. The endless controversy as to whether diphtheria is primarily a local or a general disease is settled in favor of the doctrine that it is primarily local, and that the grave constitutional symptoms are the result of intoxication with poisonous products, formed by the local action of the bacilli. We are able to study experimentally the various effects resulting from the action upon the animal body of the specific toxic products of the diphtheritic germ. We can separate the alterations belonging to the disease itself from the many complications of the disease. Intelligent measures of prophylaxis can be based upon a definite knowledge of the characters of the specific germ and its behavior in the body, and such measures have already been formulated. Rational indications for treatment can be established.

That the blood serum of animals rendered artificially immune against the diphtheritic bacillus and its products is capable of destroying or nullifying the effects of the toxic albumens of diphtheria, although not of killing the bacilli, is a new conception in medicine, and this, together with other observations of Fränkel and of Behring upon the production of immunity, opens up new vistas which may lead to results of the greatest scientific and practical importance.

THE CAUSATION OF DIPHTHERIA[1]

The subject which I have selected for this address is so largely a topic of the day, and fills at present so much space in medical journals, that the main facts which will be here presented must appear familiar to those who have followed recent medical literature. Nevertheless, many of these facts are so new and so important, and the disease to which they relate is one of such great interest, that no apology is needed for bringing before you a brief summary of recent investigations concerning the causation of diphtheria, and the light which these discoveries have shed upon our knowledge of the pathology, prophylaxis and treatment of a disease more dreaded, and justly so, than any other infectious disease always present among us. Although these new discoveries are mainly the results of bacteriological work, it will be my purpose to dwell not so much upon minute bacteriological descriptions, more suitable to the laboratory and to special articles than to a general address of this kind, but rather upon those points which illustrate the application of these discoveries to a fuller and more correct knowledge of the nature, etiology, pathology, diagnosis, prevention and treatment of diphtheria.

The history of our knowledge of diphtheria illustrates the difficulties which we encounter in endeavoring to reach a full understanding of a disease upon the basis of its symptomatology and pathological anatomy alone. Consider for a moment what are some of the uncertain and still much disputed questions concerning this disease.

Is diphtheria primarily local or constitutional in its origin? Are all pseudomembranous inflammations of the throat, not directly referable to caustic irritants, diphtheria? Is there a purely local, noncontagious pseudomembranous laryngitis called croup distinguishable from diphtheria? Are the pseudomembranous anginas secondary to scarlatina, and less frequently to measles and some other infectious diseases identical with diphtheria? Is there any relation between follicular tonsillitis and diphtheria? May diphtheria occur in a mild form as a simple catarrhal inflammation of the throat? Are pneumonia, acute nephritis, suppuration of the glands in the neck, etc., referable to the direct action of the diphtheric virus; in other words, what lesions belong to the disease and what are complications? Shall reliance be placed chiefly upon local or upon general treatment?

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[1] Annual address before the Medical and Chirurgical Faculty of the State of Maryland, Baltimore, April 29, 1891.

Tr. M. & Chir. Fac. Maryland, Balt., 1891, 242-250.

These are some of the questions which the most careful study of the clinical history and the anatomy of diphtheria has not been able to answer clearly and unmistakably. Nor are these matters only of theoretical importance. Referring to the two doctrines of the local and of the constitutional nature of diphtheria, Dr. J. Lewis Smith, one of the most painstaking and profound investigators of this subject in this country, says: "It is a matter of great importance—an importance transcending that of almost any other subject relating to the pathology of this dreadful scourge—that we should ascertain certainly and clearly which of the two prevailing theories is true, since the theory influences practice."

I hope to be able to show in the course of this address that this question, as well as others mentioned, have now been answered "clearly and certainly," and that we are in a position to solve other of the problems still remaining in doubt. All of this gratifying widening and deepening of our knowledge has come from the discovery of the microscopic germ which is the specific cause of diphtheria, and from the study of the singular and interesting properties of this germ. There is, perhaps, no other disease, with the exception of tuberculosis, upon which greater light has been shed by the discovery of its specific cause than upon diphtheria.

Permit me to present to you, in the first place, the evidence that the so-called Klebs-Löffler bacillus is really the specific agent of infection in diphtheria; and then to describe the most important properties of this microorganism, so fas as they bear upon the subject of this address.

There are three things especially which have rendered difficult the convincing demonstration that a given microorganism is the specific cause of diphtheria.

The first is the uncertainty as to what shall be called diphtheria, and the possibility that included under this name are various affections which may be due to different causes. Hence it might happen that one investigator studying one class of cases—for instance, the pseudomembranous anginas associated with scarlet fever, measles, etc.—might obtain results different from another who observed another class of cases. In view of this uncertainty, it is clear that conclusions as to the causation of primary diphtheria should be derived from the investigation of cases which all are agreed in regarding as genuine primary diphtheria. When the etiology of this undoubted class of cases has been determined, we are in a position to examine whether the secondary and other doubtful forms of diphtheria have a different causation or not.

A second difficulty has been not in the demonstration of microorganisms in diphtheria, but rather in the abundance of bacteria present in diphtheritic membranes. Of the various species of bacteria found in the false membranes of diphtheria we must determine which are constantly present and which are inconstant. If we find one species always present in large number and absent in other conditions, the presumption is that this specific cause of the disease, and this presumption becomes a certainty if we are able to reproduce experimentally, by inoculation with pure cultures of this germ, a disease in all respects identical with human diphtheria.

Just here we meet the third difficulty, which consists in bringing positive evidence that our experimental disease is in reality identical with human diphtheria. We are accustomed to regard the fibrinous pseudomembrane on the mucous membrane of the tonsils, pharynx, and air passages as the most certain anatomical criterion of diphtheria, but we have long known that it is possible to produce experimentally in animals, a variety of agencies which have nothing to do with diphtheria, false membranes histologically identical with the diphtheritic membrane. Hence the mere production of a false membrane by the inoculation of a germ suspected to be the cause of diphtheria, although it constitutes weighty evidence, cannot be considered absolutely conclusive that such germ is really the cause of diphtheria. But are there not other anatomical changes in the body characteristic of diphtheria besides the diphtheritic membrane? We owe especially to Oertel the demonstration of the fact that the diphtheritic virus is a most peculiar poison to the cells of the human body, and that it produces areas of cell death not only on the surface of the mucous membranes, but also in deeper parts, in various lymphatic glands at a distance from the local lesion and in the spleen. These changes can be demonstrated by histological examination. Now, if we find that our suspected germ, besides causing pseudomembranous inflammations, produces identical foci of cell death; if we find that it injures the kidneys, as we have long known it does in human beings; and if we find that it causes late muscular paralyses resembling the postdiphtheric paralyses which the clinician regards as of such diagnostic value in doubtful cases of sore throat, then we shall have evidence strong indeed that this germ is the real specific cause of diphtheria.

It is not surprising that all these difficulties were not overcome in the first important publications of Klebs in 1883, and of Löffler in 1884. We owe to Löffler the first bacteriological study of diphtheria by the modern culture methods. Although his work was most valuable and accurate, he was compelled to express himself reservedly as to the significance of the bacillus which we now believe to be the cause of diphtheria. No such complete chain of proof was brought forward as Koch presented in his first memorable publication upon the tubercle bacillus two years previously. Hence it came about that the Klebs-Löffler bacillus from the first assumed a doubtful status, and that even today it is often referred to in writings on diphtheria as only one among many claimants to be regarded as the cause of diphtheria.

I shall not weary you by following the historical path in which obstacle after obstacle has been removed which stood in the way of the full recognition of the Löffler bacillus as the infectious agent of diphtheria, but I shall simply direct you to the goal which has been reached.

It is now established that this bacillus is constantly present in large number in the pseudomembranes of all cases of primary diphtheria, and that no other species of bacteria is constantly to be found in this situation. The expectation expressed a year ago by Löffler that the results on this point would not be found to be different in this country from those in Europe has been fulfilled by the bacteriological examination by Dr. Abbott and myself of a series of cases of primary diphtheria occurring in Baltimore, in all of which we found the Löffler bacillus, as the only constant organism, as well as by the latest publication of Dr. Prudden. The occurrence of a bacillus, with all the properties of the virulent diphtheric bacillus, in persons not affected with diphtheria is so extremely exceptional that Löffler, notwithstanding years of searching, has been able to find it only once under such circumstances.

The Löffler bacillus can be readily obtained in pure cultivation on artificial media and its properties studied outside of the body. The sum of these properties suffices for its positive and ready identification.

Inoculation of pure cultures of this bacillus is found to be pathogenic for guinea pigs, cats, pigeons, rabbits, and some other animals. By such inoculation a disease can be reproduced identical in all essential features with human diphtheria, and there is no other species of microorganism known capable of reproducing all of these features. In suitable animals we can cause by inoculating them with the diphtheric bacillus pseudomembranous inflammations of mucous membranes indistinguishable from those of natural diphtheria, swelling of adjacent lymphatic glands, multiple ecchymoses, serous transudations, fatty degenerations and other lesions in the kidney and liver, late muscular paralyses, and foci of cell death resembling those in human diphtheria. Upon this last point the investigations in full have not yet been published, but researches have for some time been directed, in the pathological laboratory of the Johns Hopkins University, to the detection of areas of cell death in the internal organs of the animals inoculated with diphtheria, and I may say here that we have been able to confirm and extend in animals Oertel's researches on this subject in human beings.

By the labors, therefore, of many investigators since Löffler's first publication in 1884, including those of Löffler himself, all of the conditions have been fulfilled for diphtheria which are necessary to the most rigid proof of the dependence of an infectious disease upon a given microorganism, viz.: the constant presence of this organism in the lesions of the disease, the isolation of the organism in pure culture, the reproduction of the disease by inoculation of pure cultures, and similar distribution of the organism in the experimental and in the natural disease. We are then justified in calling the Klebs-Löffler bacillus, *Bacillus diphtheriae.*

Let us now turn our attention to the most important properties of this bacillus. However great may be the scientific interest of the discovery of the specific germ of an infectious disease, this discovery by itself is after all only the realization of the faith of enlightened physicians that the infectious diseases are all caused by specific living organisms. The practitioner is fully justified in inquiring how this discovery helps him to a better understanding of the disease, to a more certain diagnosis, to a more accurate interpretation of symptoms, to more effective measures of prevention and of treatment. Although these expectations have been met only in part, enough has been accomplished already to show that great practical value has come and is likely to come in still larger measure from the discovery of *Bacillus diphtheriae.*

The specific germ of diphtheria is a bacillus, devoid of independent motility, averaging in length about that of the tubercle bacillus, but thicker than this. It presents itself both in diphtheritic membranes and in cultures often in such bizarre forms that these belong to its most characteristic morphological properties. It grows upon our ordinary culture media, best upon Löffler's blood serum and bouillon mixture, but it will grow even upon steamed potato. As bearing upon a possible source of infection it is important to note that the bacillus will multiply readily in milk, without altering the appearance of the milk. It does not multiply or it does so only very sluggishly at a temperature below 18° C. (about 64° F.). It is killed by exposure for ten minutes to a temperature of 58° C. (136.4° F.). It is not necessary to describe here in detail the peculiarities of its growth upon the various culture media, although to the bacteriologist these are the guides for the isolation of the bacillus in pure culture. The series of cultures which I here exhibit to you illustrates the characteristic appearances. It suffices here to draw the conclusion that, unlike the tubercle bacillus, the bacillus of diphtheria may readily find conditions outside of the body suitable for its multiplication.

But, as is well known, it is not necessary that an infectious organism should actually propagate itself outside of the body in order to produce ectogenic infection. Such infection may occur if the organism simply preserves its vitality. On this point we know that the diphtheric bacillus, although it forms no spores, is among the more resistant bacilli of the non-spore-forming class, withstanding for a long time drying and other influences injurious to the less resistant forms. The specific bacilli have been obtained in cultures made from diphtheric membranes preserved dry in a piece of linen cloth for five months. This property is in harmony with the experience that rooms, clothing, and other objects may retain for months the diphtheric virus in an active state. As *Bacillus diphtheriae* may live for a still longer period in the moist state, and as moist, damp dwellings have long been held to be particularly favorable for the development of diphtheria, it is probable that statements as to the preservation of the diphtheric virus in such situations for a year or more are not without foundation.

Of capital importance is the establishment of the fact that *Bacillus diphtheriae* develops only locally at the site of infection, and does not invade the tissues or the circulation. It is found only in the diphtheritic psuedomembrane, and not even in the subjacent mucous membrane. Indeed, it is only the superficial parts of the false membranes which contain the bacilli. The determination of this fact gives at once a clear and decisive answer to the long mooted question as to the primarily local or constitutional nature of diphtheria. Diphtheria is, without a doubt, local in its origin. The germ which causes this disease not only makes its first appearance and multiplies where the pseudomembrane is formed, but it does not subsequently invade the blood and organs. As we shall see later, the constitutional symptoms are due to the reception of a chemical substance or substances of remarkable toxic properties, produced by the local development of the diphtheric bacillus.

This settlement of the controversy as to the local or the constitutional origin of diphtheria is one of the most important outcomes of the bacteriological study of this affection, for the question pertains to a fundamental point in our conception of the disease. Nevertheless, from the point of view from which this controversy has usually been waged, the triumph is, in my opinion, only a partial one for the localists, for as will be shown later, I believe that there are reasons as strong as ever for the employment of constitutional measures of treatment in combination with the local ones.

From what has already been said we are prepared to consider for a moment in what ways a person affected with diphtheria becomes a source of danger to those around him, and to the locality.

Bacillus diphtheriae are conveyed from the body in particles of diphtheritic exudation, saliva, and other secretions discharged through the mouth and nose. (It is not necessary to consider the comparatively exceptional localizations of the disease.) In this way the infectious substance may readily become attached to the person and clothing of the patient, and of those around him, as well as to the bedding, furniture, floor and walls of the room, dishes, and other objects.

Notwithstanding the statements current in nearly all text-books, there is no evidence that the breath of the patient contains the diphtheric germ, except as bits of false membrane or secretion may be mechanically expelled in the act of coughing, hawking, or sneezing. It has been proven experimentally that the expired air is incapable, during ordinary respiration, of detaching bacteria from the moist mucous surfaces over which it passes.

The specific germs are not so readily conveyed by air currents from a diphtheric patient to those near him, as they are, for instance, from a patient with scarlet fever, in which the germs are in all probability thrown off from the surface of the body on light epidermal scales.

Nor are the chances of infection of the sources of supply of drinking water with the diphtheric bacillus so great as is the case with such diseases as cholera and typhoid fever, in which the stools contain in large number the specific bacteria, and are likely to be disposed of in such a way that under bad sanitary conditions these bacteria may find their way into wells and streams. While there are some accounts intended to show the conveyance of the virus of diphtheria through the drinking water, we do not hear much of this as a source of infection, and it is not likely that it plays an important rôle, although it is probable that the diphtheric bacillus may sometimes be discharged by the stools.

Diphtheria is one of the infectious diseases the germs of which may be taken into the body by the inspired air. Inasmuch as bacteria cannot, under ordinary conditions, occur as floating matter in the atmosphere until they have been completely dried down so that air currents can detach as dust the little particles to which they adhere, it is evident that only those infectious germs are likely to be conveyed by the air which are not destroyed by complete drying. As has already been mentioned, the diphtheric bacillus withstands for months desiccation which is so injurious to the cholera vibrio and to many other species of bacteria. While, therefore, we must admit that air-infection with the bacillus of diphtheria is a real and conspicuous danger, it is well to bear in mind that modern bacteriology has taught us the great lesson that the most frequent and important mode of infection is by contact with infected substances. When we consider the manifold ways in which the diphtheric bacilli may be widely distributed, and when we consider the habit of young children—who are the most numerous victims of the disease—of handling everything and of putting everything into their mouths, we are led to appreciate that infection by contact must play the leading part in the transmission of diphtheria.

I will now ask your attention to a brief consideration of the most deadly of all the properties of *Bacillus diphtheriae,* namely, the power of producing extraordinary poisonous chemical substances. That the grave constitutional symptoms of diphtheria are referable to such substances has long been surmised, and as soon as it was found that the bacilli causing diphtheria develop only locally it became certain that toxic substances must be formed. The results of the investigations as to the nature and properties of these substances are not only most important for the etiology of diphtheria, but are among the most significant acquisitions of bacteriology in recent years. We owe these results chiefly to the researches of Roux and Yersin and of Brieger and Fränkel.

If bouillon cultures, four or five weeks old, of the diphtheric bacillus—preferably containing some blood serum—be sterilized by heating them at temperatures of about 55° C., or if they be deprived of bacteria by filtration through a Chamberland porcelain filter, it is found that the sterilized culture fluid possesses highly poisonous properties when injected, even in small quantity, into animals. The toxic substance is not of a crystallizable, alkaloidal nature of the kind known as a ptomaine or a toxine, for no such substance can be detected in these cultures. The poisonous material, although it has not been separated in a condition of absolute purity, has been shown to be probably of a proteid nature. It is precipitated by alcohol and by saturation with ammonium sulphate and sodium phosphate. It is mechanically dragged down by fine precipitates, such as those of calcium phosphate. It is soluble in water. Obtained in a state approaching purity it appears as a white, amorphous mass of light specific gravity. In the moist state it loses, in great part or entirely, its poisonous properties when subjected to heat above 60° C., but when dry it stands temperatures of 70° C. It is not volatile, and can be preserved for months at least in the dry state, without loss of its characteristic properties.

Some idea of the extraordinarily poisonous nature of this substance can be obtained from the statement of Roux and Yersin, that four-tenths of a milligramme of the substance (after deducting the weight of the ashes and of the non-poisonous part insoluble in alcohol) obtained by evaporating to dryness the active culture fluid, is sufficient to kill at least eight guinea pigs, weighing each four hundred grammes, or two rabbits, weighing each three kilogrammes. As the major part of these four-tenths of a milligramme is represented by other substances than the diphtheric poison, it is apparent that the poison must be of appalling potency.

If this poison, or the sterilized fluid containing it, be injected into a guinea pig or rabbit or other susceptible animal, it is found to be capable of producing all of the changes in the body which follow the inoculation of pure cultures of the diphtheric bacilli, with the exception of the pseudomembrane. For the production of this membrane the bacilli are necessary, but the other lesions, such as the local oedema after subcutaneous injections, the ecchymoses, the serous transudations, the changes in the kidney, liver, and other parts, are observed after the injection of the toxic albumin, as the diphtheric poison is sometimes called, as well as after inoculation with the bacilli. If the animals live sufficiently long, muscular paralyses may develop.

The most remarkable property of this toxic albuminoid substance, or these substances (for we do not know positively that there is only one such substance), is that when injected in a single sufficiently small, but fatal dose, it may produce no apparent disturbance for days, and the death of the animal may occur many days or even weeks, or months after the injection. This is a quality which we have not been accustomed to attribute to chemical poisons, and it is evidently one of the greatest practical significance. If it is true that the bacilli of diphtheria are capable of producing at the site of their local development poisonous substances which, when at first received into the system, may cause no manifest harm, but which may destroy life perhaps after days, then it is clear that even if we succeed in killing the bacilli in the pseudomembrane, the individual may still die of toxaemia.

That the bacilli of diphtheria may produce apparently identical toxic proteids in the animal body has been demonstrated. The poisonous proteids are probably allied in their constitution to the poisonous substances found by Weir Mitchell and Reichert in the venom of rattlesnakes and of other serpents, and belong to a group of interesting chemical products now known to be produced by various pathogenic bacteria and other organisms, and which have been and are still being carefully studied, among others, with especial success by Sidney Martin and by Hankin in England. While there is reason to believe that the diphtheric poison is a proteid this view cannot be regarded as established until the poison has been isolated in a state of purity. As we know that the poison can be mechanically dragged down by fine precipitates, it is possible that it is intimately united to the precipitates of albumose without being itself an albumose. Interesting as it might be to tarry longer at this point, we cannot complete this outline sketch of the properties of the diphtheric bacilli without the consideration of certain other important characteristics which they possess, and time admonishes us to hasten on.

It is well known that the character of the symptoms, and especially their gravity, vary in a marked degree not only in different cases of diphtheria, but also in different epidemics. There are epidemics of a mild character, and there are epidemics, such as some of those of recent years in the northwestern part of this country, which sweep away nearly all the children of a neighborhood or a town. In estimating the value of different methods of treatment, this is a point not always kept sufficiently in view. The question suggests itself whether there is anything known concerning the attributes of *Bacillus diphtheriae* which will explain these differences.

As to this point, it is to be said that this bacillus, as obtained in pure culture from different cases of diphtheria, varies in its virulence, as tested upon animals, to a greater degree than any known pathogenic organism. Diphtheric bacilli isolated from different cases of diphtheria, will sometimes kill guinea pigs by subcutaneous inoculation in twenty-four hours; in other cases they require two, three, four, even nine days to accomplish this result. From two cases of diphtheria of slight severity recently examined at the Johns Hopkins Hospital, Dr. Abbott obtained in pure culture a bacillus identical in its morphology and its behavior on culture media with the diphtheric bacillus, but incapable of killing guinea pigs by subcutaneous inoculation. In this respect it resembles the so-called pseudodiphtheric bacillus, but it differs from this organism in having been present in the diphtheritic exudate in large number.

While observations are as yet insufficient to justify a positive statement, there seems to be a general correspondence between the virulence of the bacilli and the gravity of the case in which they are found, but there are exceptions to this. It is interesting to note that as a case progresses toward recovery a tendency has sometimes been observed toward a diminution in the virulence of the bacilli. It has been demonstrated that the less virulent the bacillus, the smaller is the amount of the toxic proteid which it is capable of producing in cultures, this substance being replaced, according to Fränkel and Brieger, by another albuminoid product devoid of toxic properties. While other factors may be, and doubtless are, concerned in determining the severity of a case of diphtheria, it is evident that the demonstrated variability in virulence of the diphtheric bacillus affords an explanation of many of the differences observed in the onset and decline of an outbreak of diphtheria and in different epidemics of this disease. The existence of an attenuated variety of *Bacillus diphtheriae,* which may, under circumstances which we do not now understand, acquire virulence, is unquestionably of great significance in the epidemiology of diphtheria.

Roux and Yersin have brought forward a number of reasons for regarding the so-called pseudodiphtheric bacillus (that is a bacillus resembling, if not absolutely identical with the diphtheric bacillus, in its morphology and its behavior on culture media, but incapable of killing animals) as an attenuated form of *Bacillus diphtheriae*. In the two cases of apparent diphtheria referred to, in which a bacillus, which we could not distinguish from the ordinary diphtheric bacillus, save by absence of pathogenic properties when inoculated into guinea pigs, there was a thin, grayish pseudomembranous deposit on the tonsils, and the symptoms were of a mild character. The bacilli were present in large number and were mingled in each case with a large number of the golden pyogenic staphylococci. It would be interesting to determine whether the virulence of the diphtheric bacilli may be modified by the presence of other bacteria. Whether or not we are justified in regarding the two cases of pseudomembranous angina referred to as genuine diphtheria cannot be decided until the status of the pseudodiphtheric bacillus is settled.

I wish now to say a few words concerning the diagnostic value of the bacillus of diphtheria, and this will afford a convenient opportunity of touching also upon the existence of very mild cases of diphtheria and upon pseudomembranous anginas, which are not due to the diphtheric bacillus.

While typical cases of diphtheria, especially when occurring during an epidemic, offer no especial difficulties in diagnosis, every practitioner of experience will admit that cases occur often enough which he does not know whether to call diphtheria or not. If the case is a mild one, with whitish patches on the tonsils or pharynx, which soon disappear, and constitutional symptoms are slight, he does not generally venture to make a diagnosis of diphtheria. Still the diagnosis in just this class of cases is of great importance, for such a case may be a dangerous source of infection to others.

Now we possess in the detection of the Klebs-Löffler bacillus a positive means of diagnosis of diphtheria, and it is to be considered how far this means is available to the general practitioner. The bacteriological method of diagnosis involves, first, the microscopical examination, with an oil-immersion objective, of properly stained cover-glass specimens of the suspected inflammatory exudation; second, the preparation and study of cultures from this exudation made upon suitable media, preferable Löffler's blood serum and bouillon mixture; and third, in some cases the inoculation of a guinea pig with the pure culture. In some cases the mere microscopical examination of cover-slip preparations from the exudation may suffice, but the diphtheric bacillus cannot be identified in this way so positively as the tubercle bacillus, for instance; and in just the class of cases where doubt as to the diagnosis is likely to exist, this procedure is most apt to prove insufficient. The examination of the cultures can be made the day following their preparation, and in nearly all cases this will afford a positive answer. The preparation of the cultures requires some training in bacteriological methods, and also a modest outfit of bacteriological apparatus. To a person thus equipped the examination offers no especial difficulties. Now, although Roux and Yersin have attempted to popularize the method, and have urged its general adoption by medical practitioners, I do not think that under existing circumstances it is likely to be widely applied in general practice. The method can be readily employed in hospitals and children's asylums, where it is calculated to be of great service, and in most large cities probably some one will be found who can be called upon to conduct such examinations. As the importance of practical training in bacteriology becomes more widely recognized in medical teaching, a larger number will enter upon the practice of their profession prepared to employ bacteriological methods of diagnosis.

Bacillus diphtheriae has been found in a number of cases of inflammation of the tonsils and pharynx in which the exudation and the symptoms were not sufficiently characteristic to permit a positive diagnosis in any other way than by the recognition of the specific bacillus. We do not possess sufficient information to be able to say how frequent these cases, which are generally of a very mild character, are, or how they can be diagnosed by a merely clinical or anatomical examination. That they are not infrequent during the epidemic prevalence of diphtheria seems probable from clinical experience.

The view which has been widely advocated, that most cases of so-called follicular tonsilitis, which, as is well known, may prevail as an epidemic and may present marked but usually not dangerous constitutional symptoms, is a form of diphtheria not supported by bacteriological examination. In these cases the diphtheric bacillus is usually absent, but under the rather vague name of follicular tonsillitis are doubtless included some cases of genuine diphtheria, generally mild, but occasionally developing into unmistakable diphtheria.

Not all pseudomembranous inflammations of the throat are caused by the specific bacillus of diphtheria. It has been demonstrated by Wurtz and Bourges that this bacillus is absent, and that a streptococcus is present in nearly all cases of pseudomembranous angina developing in the early stages of scarlatina, so that most cases of so-called scarlatinal diphtheria are not diphtheria at all, if we limit, as seems best, the term diphtheria to the affection caused by the Löffler bacillus. The Löffler bacillus is, however, present in the diphtheria appearing in the late stages of scarlatina and during convalescence, and it seems that scarlet fever is a predisposing cause of the development of genuine diphtheria.

In a series of cases, numbering 24, of pseudomembranous inflammations of the tonsils, pharynx and larynx, designated at the time as diphtheria, Dr. Prudden found in all but two a steptococcus, and in none the Löffler bacillus. These cases were, for the most part, either secondary to scarlatina, measles, whooping-cough, or erysipelas, or developed under the same epidemic influence as these diseases, so that these cases probably should not be regarded as genuine diphtheria. This is the view now held by Dr. Prudden himself in his latest publication, according to which renewed observations in cases of primary diphtheria have convinced him of the causative significance of the Löffler bacillus. Roux and Yersin, whose work has contributed largely to the present recognition of the Löffler bacillus as the cause of diphtheria, have also examined with negative result, so far as this bacillus is concerned, a few cases with pseudomembranous deposits on the tonsils and pharynx of such a character that the clinician had no doubt of the existence of diphtheria. We must admit, therefore, that pseudomembranous inflammations of the throat resembling diphtheria occur, both secondary to infectious diseases and apparently primary, in which the Löffler bacillus is not found. The further etiological study of these cases and their more precise characterization as to clinical history and pathological anatomy constitute a promising field of investigation. Until certain points have been cleared up by such investigation, there may be a certain amount of confusion as to the conception of diphtheria, but this affection seems to be even now sufficiently well characterized as to its symptomatology, pathological anatomy, and etiology to constitute a definite and independent disease which need not be confounded with other diseases.

A few words may be appropriately introduced here as regards the use of the terms diphtheria and diphtheritis. The term diphtheritis or diphtheritic inflammation is used in an anatomical sense to designate a certain kind of pseudomembranous inflammation of a mucous membrane which may be produced by a variety of causes. The designation of certain other varieties of pseudomembranous inflammations of mucous membranes as croupous and as pseudodiphtheritic still further complicates the terminology. It is now generally recognized that it is best to limit the use of the word diphtheria to a definite disease and not to an anatomical process, thus making a distinction in the employment of the terms diphtheria and diphtheritis, the former being applied only to the disease due to a specific cause and the latter to an anatomical condition due to a variety of causes, of which the specific cause of diphtheria is only one among many. The adjective derived from diphtheria would be diphtheric, and that from diphtheritis diphtheritic. The disease diphtheria may appear in the form of each or all of the varieties of pseudomembranous inflammation mentioned. It has for some time been clear that the boundaries of the disease which should be called diphtheria cannot be sharply drawn save on etiological grounds. So long as these grounds were lacking the boundaries were uncertain, vague, and fluctuating. We now possess definite criteria for the recognition of the disease, and it seems to me that instead of saying that diphtheria may be caused by a variety of microorganisms, a statement which is undoubtedly true if we understand by diphtheria all pseudomembranous inflammations of the throat, it conduces to clearness and definiteness to confine the term diphtheria to the disease caused by the Klebs-Löffler bacillus. This will not necessitate material deviation from ordinary usage. The etiological basis of classification of disease is the surest and best, and in this case it will doubtless be found to correspond to a symptomatic and anatomical entity.

The data at present at hand are insufficient to settle the burning question as to the existence, independent of diphtheria, of a disease called membranous croup, supposed to be of a purely local, non contagious character. Not a few cases of pseudomembranous laryngitis without membranes on the tonsils or pharynx have been examined with the result of finding the Löffler bacillus. One such case, diagnosticated by the physician as croup, will be found in the series reported by Dr. Abbott and myself. In this we detected in large numbers the diphtheric bacilli on the mucous membrane of the pharynx, although no membranous deposit was visible, and similar results have been recorded by others, so that there is no doubt that diphtheria may occur with membranous inflammation limited to the larynx. In Dr. Prudden's first series of cases there were some instances of pseudomembranous inflammation confined to the larynx and trachea in which steptococci, but no Löffler bacilli were found, so that it is probable that pseudomembranous laryngitis, without involvement of the tonsils or pharynx may be produced by the streptococcus. But whether or not membranous croup may occur in a sense advocated as a purely local, noncontagious affection cannot be said to have been settled by bacteriological investigations.

An account of the results of the bacteriological study of diphtheria would be incomplete without some reference to pathogenic bacteria, which are frequently, although not constantly found associated with the Löffler bacillus in diphtheric exudations. These associated bacteria may be the cause of grave complications. Diphtheria is no exception to the rule that all necrotic and ulcerative processes on mucous surfaces where bacteria normally occur open the way to the invasion of pathogenic bacteria, especially to the widely distributed pyogenic streptococci and staphylococci. Mention has already been made of the probable causation of certain secondary pseudomembranous anginas by a streptococcus. The same or apparently the same streptococcus is very commonly present in diphtheria. It is usually held to be identical with *Streptococcus pyogenes*. This organism, unlike the Löffler bacillus, is capable of invading the blood and tissues. The most frequent and important complication produced by this streptococcus in diphtheria is bronchopneumonia, which develops as an aspiration pneumonia, as has been shown by the investigations of Prudden and Northrup. The common pus-producing bacterium, *Staphylococcus pyogenes aureus* is, next to the streptococcus, the most common pathogenic bacterium complicating diphtheria. It is to the invasion of these secondary bacteria that such complications of diphtheria as acute ulcerative endocarditis, suppuration of lymphatic glands, inflammations of serous membranes, and erysipelas are referable.

A matter which, although not new, has attracted much attention in recent years, may prove to be of such importance in the etiology of diphtheria that I will direct your attention to it for a few moments. Evidence has been brought forward intended to show that diphtheria may be communicated to human beings by domestic animals afflicted with this disease. The animals chiefly concerned are cattle, cats, and fowls. There have been reported in England during the last decade epidemics of diphtheria in which the evidence is strong that the diphtheric germ was conveyed in milk. There are two theories as to these milk epidemics. One is that the diphtheric virus got into the milk from persons affected with diphtheria, the other is that the cows yielding the milk were affected with diphtheria. This second daring hypothesis Klein has attempted to support by experiment. He claims that by the inoculation of two cows subcutaneously on the shoulder with a broth culture of *Bacillus diphtheriae* he has succeeded in producing genuine infection with the appearance of vesicles and pustules on the udders and the elimination of the specific bacilli by the milk. These experiments should be received with great caution, as they are in opposition to all that we know concerning the exclusively local development of the bacilli at the point of inoculation. We possess no satisfactory evidence that cattle are ever affected with a natural disease identical etiologically with human diphtheria, although it is known that an affection sometimes called diphtheria may appear in calves.

That cats may acquire diphtheria and may be a means of transmitting the disease to human beings is a widely-spread belief. Medical literature contains many instances in which on the one hand, cats appear to have contracted a disease by eating substances contaminated with the discharges from diphtheritic persons; and, on the other hand, children seem to have become infected with diphtheria by handling sick cats. Noah Webster, in his curious book on "Epidemic and Pestilential Diseases," published at the end of the last century, noted the coincidence of cat distempers with malignant angina. In the recently published "Annual Report of the Local Government Board of England," Klein has brought together the evidence to be found on this point. Inasmuch as cats are among the animals most susceptible to inoculation with cultures of the Löffler bacillus, acquiring a disease resembling human diphtheria, there is no *a priori* reason why they may not be the subjects of a natural disease etiologically identical with diphtheria. But the possibility of the experimental production of a disease in an animal is no proof of the natural occurrence of such disease; and thus far there is not satisfactory evidence that diphtheria occurs as a natural disease in cats. What is necessary to settle this question is to make careful bacteriological studies in cases of suspected diphtheria in cats. The matter is of sufficient interest and importance to merit careful study, and we should be glad of the opportunity of making examinations in suitable cases which may come to the notice of physicians in this vicinity.

Pigeons also are susceptible to inoculation with the Löffler bacillus, and it is well known that a membranous inflammation of the mouth, fauces, and trachea occurs as a destructive epidemic in chickens, turkeys, pigeons and other birds. This so-called diphtheria of fowls has not, however, been found to be caused by the same microorganism which causes human diphtheria, so that notwithstanding some rather striking observations according to which human beings have appeared to contract diphtheria from fowls, there is no positive proof of the dissemination of genuine diphtheria in this way. It may be that human beings may become infected by the germs producing the so-called diphtheria in domestic animals, but if so, there is no proof that the disease produced is etiologically identical with the disease caused by the Löffler bacillus.

We have now passed over, gentlemen, in rapid survey, some of the leading factors concerned in the causation of diphtheria. The limits of an address do not permit an exhaustive consideration of the subject even from the bacteriological point of view. Many circumstances of great importance in the etiology of diphtheria, such as the influence of unsanitary surroundings, particularly those of a domestic character, and the often dominant rôle of school attendance upon the dissemination of the disease could not be considered. These, however, are etiological factors which can be more profitably discussed from an epidemiological than a bacteriological point of view.

I have already made such demands upon your patience that I have not left myself sufficient time for any adequate consideration of the prophylaxis and treatment of diphtheria—subjects, indeed, which would furnish ample material for a separate discourse. I am glad to see that the subject of the treatment of diphtheria is to be presented in a special paper before this meeting. I cannot refrain, however, from directing your attention to a few of the general principles controlling methods of prevention and of treatment of diphtheria, and from pointing out the directions in which our increased knowledge of the causation of diphtheria leads us in our search for more effective measures to these ends.

The very fact that we are no longer groping in the dark, but that we know the enemy, his strength and his weakness, should inspire our courage and hope, should make us forge new weapons and sharpen our old ones, and should point out where to ward off attack and where to force the battle.

The possibility of making an early diagnosis of diphtheria by the aid of bacteriological examinations enhances the chances of success in coping with this disease, both in prevention and in treatment, and, as already intimated, provision should be made in hospitals and children's asylums for making such examinations.

Inasmuch as the diphtheric bacilli are present only in the false membranes and other local products of the disease at the site of infection, and are distributed outside of the body primarily through these, it is apparent that the patient should be strictly isolated; that unnecessary fabrics and other objects, especially such as cannot be readily disinfected, should be first removed from the room where the patient is placed; that care should be taken to prevent, as far as possible, the soiling of the person and clothing of the patient, of the attendants, and of the physician, as well as that of other objects in the room, with the discharges of the patient; that opportunity should not be afforded for the desiccation of these discharges, which then may contaminate the air, and that efficient measures of disinfection of the room and of all objects which by any possibility can become infected should be employed. That the enforcement of the measures indicated is capable of restraining the spread of the disease, even in crowded infants' asylums and hospitals, has already been demonstrated by actual experience. They require for their accomplishment education on the part of the community, of physicians, and of sanitary authorities in the principles of disinfection, and an intelligent appreciation of the dangers to be guarded against. I seize this occasion, as I have others, to urge the importance in cities of public disinfecting establishments, constructed according to improved modern principles, and of a corps of men in the employment of boards of public health, trained in proper methods of disinfection.

The length of time that the patient should be quarantined depends evidently upon the duration of the period in which active diphtheric bacilli remain on the mucous surfaces attacked. As to this point we possess some definite information, which shows that the period varies within wide limits. In some cases the bacilli can no longer be found after the false membranes have completely disappeared. In many cases they vanish within three or four days after the local inflammation has subsided, but they have been found as long as fourteen days after the inflammation has gone, and when the mucous membrane appeared healthy. In a case reported by Löffler, the bacilli were found three weeks after the return of the temperature to the normal, and were present for a month altogether in a state capable of carrying infection. It is evidently not possible to set a precise limit for the period of isolation of the patient. Löffler suggests that the patient should not be permitted to mingle with others or to return to school for at least eight days after the disappearance of all local manifestations, and he reckons four weeks from the beginning of the disease as the period for keeping the children out of school. Where it is possible to do so, the length of this period can be controlled by bacteriological examinations of the mucous membrane of the throat.

We have thus far considered the patient as the immediate source of infection. The evidence already mentioned in favor of the occasional dissemination of the diphtheric virus through the milk suggests at once the importance of controlling, not only the condition of the milk as received for distribution, but also of inspecting the sources of milk supply. Evidently milk should not be permitted to be sold from dairies attached to households where there are cases of diphtheria.

It does not seem to be permissible to throw out altogether the possibility of the conveyance of diphtheria by domestic animals, especially by cats, which are likely to be fondled by children even more where the animals are sick than when they are well.

In considering what can be done to render children less vulnerable to diphtheria, and to ward off an attack after exposure to the disease, the question arises, what conditions of the individual we may regard as predisposing causes. Clinical experience, as well as experiments upon animals, indicate that morbid states of the mucous membrane of the throat, such as ordinary catarrhal inflammations, swollen tonsils, sensitiveness to "catching cold," the existence of measles and scarlatina, are predisposing factors. At all times, but especially during the epidemic prevalence of diphtheria, it is important to hold in check these morbid states as far as possible. Experiments upon animals would seem to indicate that some lesion of the mucous membrane is essential for the lodgment and multiplication of the diphtheric bacilli, but it would be entirely unjustifiable to transfer directly to human beings the results of these experiments without warrant from clinical experience, and while this experience shows the importance of such lesion, it also shows that perfectly healthy children may be attacked by diphtheria, so that we have no right to assert that in human beings infection with the diphtheric bacilli cannot occur without preexisting alteration of the mucous membrane. The frequency and readiness with which the tonsils become the seat of the diphtheric process may be explained by the peculiar anatomical structure of these parts, as has been shown by the researches especially of Stöhr and of Hodenpyl. The rarefaction of the covering epithelium and the emigration of leucocytes to the surface found as a normal condition, seem to render the tonsils particularly vulnerable to the lodgment of the diphtheric bacilli; and the lymphatic structures, here normally laid almost bare, are favorable for the absorption of the toxic products of the bacilli, as has been pointed out in the recent interesting paper of Hodenpyl, " On the Anatomy and Physiology of the Faucial Tonsils."

The prophylactic value, in persons liable to exposure to diphtheria, of cleanliness of the teeth and mouth and of the frequent use of weak antiseptic mouth washes, nasal douches, and gargles, is worthy of the attention of physicians. For this purpose Löffler recommends aromatic waters, weak sublimate solutions [1:10000 to 1:15000], or perhaps better, solutions of mercuric cyanide [1:8000 to 1:10000] also, chloroform water, chlorine water [1:1100] thymol, [1:500 parts of 20 per cent alcohol]. The use of some of these solutions evidently involves danger of poisoning in children unless special precautions are taken.

The suggestion recently made by Dr. Jacobi, that, in addition to caring for those sick with diphtheria, places of refuge should be provided for the temporary stay of children sent from home to escape infection, seems practical and calculated to meet the circumstances of many families.

The dominant rôle often played by schools in the spread of diphtheria throughout a community renders especially urgent the introduction of a system of daily medical inspection of the schools.

The importance of letting air and sunlight into dark, damp dwellings, and of attending in general to matters of domestic sanitation, is a lesson plainly to be drawn from the history of such places as nests of diphtheria.

If the researches relating to the etiology of diphtheria which have been so fruitful in recent years have not added to the lists of agents—whose name is legion—employed in the treatment of this disease, they have given precision to the indications for treatment. From what has been said concerning the localization of the diphtheric bacilli, and the peculiar characters of the toxic substances produced by them, it is apparent that the strongest indications exist, on the one hand, for the earliest possible local treatment by germicides, and, on the other, for the destruction of the poison in the system, and, in the absence of any antidote, for supporting measures of treatment which shall enable the body to withstand the effects of the poison.

The superficial situation of the specific bacilli in diphtheria seems to render this disease especially favorable for effective local antiseptic treatment, but the difficulties encountered in actually destroying the germs in the diphtheric deposits, even when these are accessible to local applications, emphasize the fact that the obstacles to thorough disinfection within the human body are exceedingly great. Many of our most powerful antiseptics, when they come into contact with the tissues and fluids of the body form precipitates which render them largely inert, and for this and other reasons they penetrate but slowly or not at all beyond a very superficial extent. But above all we are hampered by the fact that nearly all disinfectants are many times more poisonous to the cells of the human body than they are to bacteria.

But notwithstanding the difficulties mentioned, and still others suggest themselves, it has been shown both by bacteriological examinations and by clinical experience that local disinfectant applications in diphtheria are useful and may cause the specific bacilli to disappear. An important experimental study of the action upon the diphtheric bacilli of many germicides and agents used in treating diphtheria has been recently published by Löffler, who endeavored to imitate as far as possible in test tube experiments the conditions present in the mucous membrane when affected with diphtheria. I can only refer you to Löffler's work and his recommendations. Any attempt to consider here therapeutic agents would lead us far beyond the limits of our subject as well as of our time.

Even if it were within the power of the physician to bring about the complete destruction of *Bacilli diphtheriae* after the disease has taken a firm hold, he would nevertheless in many cases be called upon to combat the effects of the toxaemia resulting from the absorption of the toxic albumen already produced by the bacilli. In this respect there is an analogy between diphtheria and tetanus. In both of these diseases the specific bacteria develop only locally at the site of infection, where they produce substances of extraordinary poisonous power which enter the circulation. In the case of tetanus we are familiar with the fact that even the complete removal of the infected part will not prevent a fatal issue after a certain amount of poison has been produced and has entered the system. Experiments already mentioned have shown that animals may be killed by the injection of an amount so small of the toxic albumen produced by the diphtheric bacilli that for several days no symptoms are manifested. The physician certainly cannot neglect the dangers of intoxication by such a substance as this.

The interesting experiments of Behring and Kitasato upon the production of immunity against tetanus and diphtheria encourage us to hope that we may in time come into the possession of direct antidotes to the chemical poisons of these diseases. These experiments have shown that the blood serum of animals naturally or rendered artificially immune against diphtheria, possess the remarkable property of neutralizing or destroying the toxic substances produced by *Bacilli diphtheriae,* and that immunity depends upon this property. The same is true of tetanus. Whether or not any therapeutic application in human beings can be successfully made of this property of the blood of immune animals, has not yet appeared.

Failing any such antidote, it should be the aim of the physician to assist nature to withstand the poison by supporting the strength of the patient in every way possible by remedies and food.

I have attempted, gentlemen, in this address to show, although necessarily in an imperfect and fragmentary manner, that the discovery of *Bacillus diphtheriae* and the study of its properties have rendered our knowledge of diphtheria in many ways more accurate and fuller, have settled many questions of controversy, and have dissipated some errors. The new light has not yet penetrated into all of the dark corners, but when we consider what has been gained in less than a decade, we are justified in expecting that here also obscurity will disappear, and that diphtheria will become in all respects one of the best understood, and, we may hope, one of the most successfully combated of the grave infectious diseases.

THE HISTOLOGICAL CHANGES IN EXPERIMENTAL DIPHTHERIA[1]

(From the Pathological Laboratory of the Johns Hopkins University and Hospital)

The well known observations of Oertel concerning peculiar histological changes in internal organs in human diphtheria, make it important to determine whether similar alterations occur in animals inoculated with the Klebs-Löffler bacillus of diphtheria. If such changes are found in the latter cases, they add valuable proof to the demonstration that the experimental is essentially identical with human diphtheria.

Microscopical changes in the internal organs, especially in the liver and kidney, in cases of experimental diphtheria have been observed by Löffler, Klein, Beck, and others. The fullest description thus far given of such changes is by Babes,[2] whose investigations on this point relate chiefly, also, to the liver and kidneys of rabbits. The changes in the liver observed by Babes varied in different cases. The principal lesions in this organ noted by him are swelling and degeneration of the liver cells, as well as proliferation of these cells, accumulation of leucocytes, often with fragmented nuclei (which, in some cases at least, he regards as evidence of proliferation), and of hyaline material and yellowish granular substance in the blood vessels, and swelling and proliferation of the endothelial cells of the capillaries. In the kidneys he observed parenchymatous swelling of the epithelial cells, proliferation and desquamation of these, as well as of endothelial cells, and hyaline masses in the blood vessels. In the spleen he found hyperplasia of the lymphatic apparatus, and in one case he speaks of numerous nuclear fragments in the venous lacunae.

Our study has been conducted with the view of ascertaining the histological changes in all of the organs of animals dead of experimental diphtheria. In the course of our work we have used guinea pigs, rabbits and kittens.

At the present time our experiments are not so complete as we wish to make them, but our results have been so uniform that we feel justified in presenting a brief preliminary account of them to the Society.

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[1] William H. Welch and Simon Flexner. Preliminary communication before the Johns Hopkins Hospital Medical Society, May, 1891.

Johns Hopkins Hosp. Bull., Balt., 1891, II, 107-110.

[2] Babes: Virchow's Archiv., Bd. 119, p. 468.

The cultures used in our work were obtained from undoubted cases of primary diphtheria occurring in Baltimore, and they were in all cases pure cultures of the Klebs-Löffler bacillus.

The inoculations were made into the subcutaneous tissue of the belly wall, a site being chosen midway between the axillary and inguinal regions. There was one exception to this rule, in the case of a kitten on which tracheotomy was performed and the inoculation made into the trachea.

Death occurred in the animals experimented upon in the following lengths of time:

Guinea pigs—shortest period, 38 hours; longest period, 5 days. Rabbits—shortest period, 48 hours; longest period, 8½ days. Kittens—shortest period, 43 hours; longest period, 5½ days.

The *gross anatomical lesions* which we observed corresponded for the most part with those described by other investigators. At the seat of inoculation was a grayish, necrotic, pseudomembranous focus, and surrounding this a deep red zone of varying size; underlying it the muscle of the belly wall was greatly congested.

The subcutaneous tissue on the side of inoculation was oedematous beginning at the seat of inoculation and extending over the entire side, often into the neck, and in some cases beyond the median line to the opposite side. The axillary and inguinal regions and the place immediately around the seat of inoculation were principally affected, and the oedema often assumed an exquisite gelatinous appearance.

In some cases this oedema was composed of pure yellowish serum, and in others it consisted of serum stained with blood. The subcutaneous lymph glands of the axillary, inguinal and cervical regions were haemorrhagic and swollen. The swelling affected the glands most on the side of inoculation; but the glands of the opposite side were reddened and sometimes enlarged. We would call attention to the ease with which moderate glandular enlargement in such small animals may be overlooked, unless the very small size of the normal glands be kept in mind.

The cavities of the body showed alterations. In some cases the peritoneal cavity contained an excess of fluid—as much, sometimes, as five or six c. c. Both the visceral and parietal layers of peritoneum were much injected, and ecchymoses were present in some cases. The mesenteric glands and retroperitoneal glands were enlarged, and sometimes distinctly reddened.

The organs were not unaffected. The liver was congested: at times evidently fatty. In guinea pigs, especially, the liver showed numerous macroscopic dots and lines on the surface and penetrating the substance of the organ. In a less degree this was true of kittens also. These dots varied in size from a pin point to a pin head; sometimes they were even larger.

The lines were two mm. in length, or less. They appeared white, and did not project above the surface of the capsule.

Though the spleen was not perceptibly enlarged in all cases, yet in some it was markedly enlarged. There was considerable variation in this respect. Its color varied: in guinea pigs there was no striking alteration, while in kittens the spleen was dark, often bluish black.

The kidneys were moist and hyperaemic.

The adrenals always showed an intense congestion, and in many instances were haemorrhagic.

The intestinal lymphatic apparatus showed interesting alterations. The agminated glands of the caecum and the ileum in guinea pigs and kittens were abnormally prominent. They projected above the surface of the bowel, and exhibited a number of whitish points imbedded in a grayish material.

The pleural cavity contained in many cases an excess of fluid, amounting, at times, to three or four c. c. The mediastinal glands were swollen and reddened, and the bronchial glands likewise. The heart appeared normal, while the lungs sometimes showed areas of congestion, and even of consolidation. The deep glands of the neck were often reddened, and the thyroids were uniformly congested, sometimes haemorrhagic.

The *microscopical study* of the organs was made in the fresh state by means of frozen sections, and, again, after hardening in different ways; alcohol, Flemming's solution and Mueller's fluid having been used. The hardened tissues were stained with various aniline dyes, haematoxylin and carmine. The tissues hardened in alcohol and stained with fuchsin or methylene blue, gave, as a rule, the most satisfactory results, other methods being useful for developing certain points.

Seat of Inoculation.—Bacilli of diphtheria were found regularly in the seat of inoculation. They were present in the gray necrotic looking focus both free and within leucocytes. They may be absent from the oedematous fluid at a distance from this focus. Many of the leucocytes showed a fragmentation of their nuclei. Sections made from the seat of inoculation showed the bacilli in great numbers. The sections stained by means of Weigert's fibrin stain exhibited in a striking manner the bacilli and the fibrin.

The local action of the bacilli is of the most intense character. There is emigration and great destruction of leucocytes shown by disintegration of their nuclei; the fixed cells of the part have undergone a similar fragmentation, nuclei of connective tissue and muscle have succumbed, and leucocytes have wandered into these areas, many of the latter being destroyed also. There would appear to have been a proliferation of muscle nuclei in places not all of which have become fragmented.

Lymphatic Apparatus.—The lymph glands of the axillary and the inguinal regions were greatly affected. The changes in these structures are very typical.

There are hemorrhages under the capsule and into the substance of the gland. The blood vessels here as elsewhere in the body contain a greatly increased number of leucocytes.

The cells of the gland are the seat of great changes. The principal lesions are in the lymph follicles, the lymph cords and lymph sinuses being also affected, though in less degree. Different follicles are affected in different degrees, and there are variations in different animals even of the same species. But in no instances were the lesions entirely absent.

The lesions consist of a marked alteration in the number, character size, staining capacity and configuration of the nuclei making up the parts affected. The cell bodies are altered also, and an increased number of cells differing from the lymphoid type are found. The first thing that attracts attention is the unusual number of deeply staining bodies in the tissue. These bodies are observed to vary in size and shape, and under a sufficiently high-power some of them are recognized as nuclear figures. They are usually, however, globular, and under a magnifying power of four hundred range from fine dust-like particles to larger particles, appearing with this power the size of a pin's head. The finer particles are often aggregated into larger globular masses, which are now free, and now enclosed in cells.

There are, again, deeply staining particles present which show decided bizarre forms. Imperfect crescents, flask-shaped, bladder-like, whetstone, angular, and dumb-bell forms are more or less common. Occasionally, nuclei appear as if one end were drawn out and constricted into a ball-like protuberance that is being pinched off. The globular particulars are, at times, grouped together with the bizarre forms into larger masses; what particularly distinguishes these bodies from the normal nuclei which remain is the intensity with which they stain.

All the chromatin particles, as before mentioned, are not within cells; indeed, as a rule, they do not occur in cells, though the number within cells varies considerably. In some glands much of this material is contained within globular cells several times larger than the lymphoid cells. In some sections these cells are observed to be present in considerable numbers, partly devoid of stained particles, or nearly so, but usually they are full of the nuclear detritus.

In certain spots there is almost an absence of stained cells and particles. In these places it is possible to distinguish outlines of cells, then a finer granular, somewhat refractive, at times reticulated material, and here and there a deeply stained particle. But there are always a few cells remaining that stain more or less, and among these are round cells, larger than the lymphoid cells. This portion stains with Weigert's fibrin stain in such a manner as to indicate that the granular material is largely made of fibrin or of a substance allied to fibrin.

In the lymph sinuses, especially, occurred a considerable number of round or slightly oblong cells, quite colorless in appearance, containing bodies which resemble in a striking manner red blood corpuscles, and these cells remind one of the red blood corpuscle carrying-cells of the typhoid spleen. In alcohol preparations it was impossible to determine the exact nature of the contents of these cells. Similar cells were found in the mesenteric glands and the spleen; in osmic acid preparations, on the other hand, they are shown to be red corpuscles.

An alteration similar to that just described is found throughout the lymphatic structures of the body: in the spleen, mesenteric glands, retroperitoneal glands, intestinal lymphatic apparatus (Peyer's patches, solitary follicles, and diffused lymphatic tissue), the bronchial glands, mediastinal glands, and cervical glands. The only variation is one of degree. As far as our study has gone every gland examined has been more or less affected. The spleen is often very rich in nuclear fragments and foci of coagulation necrosis.

The cells of the *intestinal villi* in guinea pigs and rabbits and the epithelium of the intestinal mucous membrane were involved. The lesions in these structures showed a like tendency to vary in intensity. The most striking changes consisted in a fragmentation of the nuclei of cells in the villi, especially of those surrounding the central vessels (lacteals or bloodvessels), a disappearance of a large number of cells, and the presence of large round cells, similar to those described in the parts of the lymph glands most affected. These larger cells often showed a very slight staining power and shadows of cells were not uncommon. The nuclei of the epithelium were distinctly and extensively fragmented. Nuclear figures were to be seen and saphrophytic bacteria were found in the necrotic tissue. These fragmented nuclei partook of the same characters as those already described and exhibited the same intense affinity for staining agents.

The Liver.—On frozen sections the dots and lines resolve themselves into smaller and larger masses of highly refractive cells, usually devoid of nuclei and retaining the outlines of liver cells. Reagents do not affect them to any great extent; acetic acid causes them to swell somewhat and to become slightly less refractive. These cells are hyaline and represent foci of dead liver cells. Besides the groups which were visible to the naked eye as lines and dots there are small groups and single cells which show the same change.

Sections of the hardened organ were studied. Those from alcohol were stained in methylene blue and eosine. By this method the areas of dead liver cells stand out clearly in red. The cells generally have well stained blue nuclei and lightly stained red cell bodies. But the hyaline cells are intensely red, either devoid of nuclei or, when a nucleus is present, it is manifestly altered. It is shrunken, often irregular in shape, and it stains differently from the nuclei generally.

Leucocytes are commonly found in such foci of dead liver cells. They are between the hyaline cells and sometimes apparently within them. There are fragments of nuclei in these places, some of which at least have been derived from the leucocytes which have wandered in.

An important change could be made out in these livers in connection with the central veins of certain lobules. Haemhorrhages under the capsule were quite common, and in these lobules deepseated haemorrhages into the tissue had taken place. These varied greatly in size, some being very small while others were quite large, involving the greater part of the lobules. It was observed that such intralobular haemorrhages originated in the central veins, and seemed to be due to a breach of continuity in their walls. For, when they were small a minute defect could sometimes be seen, and where they were larger a considerable rent was present in the vein wall through which the blood had escaped. The walls of these veins were quite refractive in the fresh state, and gave, after hardening, a quite characteristic staining for hyaline with picric acid.

This focal death of liver cells just described was observed in guinea pigs and kittens especially. In rabbits, in one case in particular, there was an extensive diffuse degeneration of liver tisue. The liver was yellowish and evidently fatty, and on frozen sections it proved to be the seat of a most extensive fatty degeneration. The cells, after the tissue had been hardened in alcohol, stained very imperfectly, many not at all. The nuclei of the liver cells were in many places in a state of disintegration or fragmentation; a few, of division. The cells throughout entire lobules were effected. Leucocytes were numerous, both in the tissues and in the vessels, and many of those in the latter were fragmented.

The Kidneys.—Fresh frozen sections showed in guinea pigs and rabbits fatty changes in the epithelium of the tubes and glomeruli In kittens, owing to the large amount of fat in the epithelium normally, it is not easy to make out a pathological increase. Yet there would appear to be an absence of fat in the epithelium of the glomeruli and collecting tubes in the cortex under normal conditions. In some of our cases these structures contained minute fat drops. A more important change is a hyaline alteration of the glomerular capillaries and the smaller arteries. This was noticed in kittens in particular; and it often affected a few loops of glomerulus, without involving the entire mass of capillaries. Hyaline substance was found completely filling the lumen of some capillaries.

Hardened and stained sections showed besides the hyaline change just mentioned fragmentation of the nuclei of the glomerular and tubal epithelium on a small scale, and the presence of minute deeply staining round bodies between and in the tubal epithelium.

Adrenals.—These were generally congested, haemorrhages were often present, and in two cases the medullary cells were distinctly hyaline.

Lungs.—There were often haemorrhages under the pleura. The blood vessels of larger size are distended with blood, and the number of leucocytes in them increased. The capillaries in the walls of the alveoli are distended with blood likewise, and there are haemorrhages into the alveoli. There is an exudation into some of them consisting of leucocytes and fibrin; but the leucocytes are few in number. There are many large cells present having a round nucleus of considerable size which stains quite deeply, but not solidly, with fuchsin. These cells are present in the areas of consolidation, and they are found also in considerable number in the nodes of the alveoli, that is, the points at which several of them come together. They are probably derived from the epithelial cells of these structures.

Fragmentation of nuclei is not a prominent feature in the lungs. It does occur, but not extensively. It was noticed in the areas of exudation, where it affects the nuclei of the large round cells as well as the leucocytes. Then it is seen in the epithelial lining of the larger bronchi, and in those bronchi that contain leucocytes fragments of nuclei are present in their lumina amid the exudation.

The *heart* was the seat of a fatty degeneration, more or less intense, in nearly every case. In many of them every muscle fibre seemed to have undergone a minute fatty metamorphosis. In others the process was less general.

Another change was noticed in the nuclei of the *muscle* fibres in certain cases. It consisted of a fragmentation similar to that in other parts.

The muscles adjoining the lymphatic glands of the axillary and inguinal regions showed degenerative changes. There was necrosis of muscle fibres and wandering in of large numbers of leucocytes, a myositis being the result.

Cultures were made from the blood, liver, kidney, and spleen of these animals with negative results in every instance.

THE HISTOLOGICAL LESIONS PRODUCED BY THE TOXALBUMEN OF DIPHTHERIA[1]

In a preliminary communication presented to the Johns Hopkins Hospital Medical Society, and published in the Johns Hopkins Hospital Bulletin, No. 15, August, 1891, we called attention to the histological changes in the organs of animals which had died of experimental diphtheria, following the inoculation of pure cultures of *Bacillus diphtheriae.* Since then we have extended our investigations so as to include the study of the lesions produced by the inoculation of the toxic products of the diphtheria bacillus. This study virtually finishes the work we have undertaken, and it is hoped soon to publish our results in detail.[2] However, in order to make our preliminary communication complete, we append this report.

The toxic products of the diphtheria bacillus with which we have operated were obtained by filtering through a new and sterilized Chamberland filter a culture of the organisms in glycerine bouillin several weeks old. The fluid so obtained was tested by means of coverslips and inoculations on glycerine agar, and proved to be sterile.

Guinea pigs were used for the experimental inoculations. The sterile culture fluid was introduced subcutaneously into the tissues of the belly-wall. The method pursued will be given in connection with the case of which the lesions are to be described. This guinea pig received on the 10th of December, 1891, 1 c. c. of the filtrate. Not having succumbed, on December 14 it received 2 c. c. more. The animal died on January 5, 1892, the duration of life since the first inoculation having been three weeks and five days, and since the last, three weeks and one day.

At the autopsy the vessels of the subcutaneous tissues were injected, and haemorrhage had taken place into the tissues of the axillary and inguinal regions. The subcutaneous tissues were moist, but there was no actual oedema present. Neither was there a visible area of localized inflammation. There was no microscopical examination made, however. The lymphatic glands of the axillary and inguinal regions were enlarged and reddened: the cervical lymph glands were swollen, and the thyroid gland was greatly congested.

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[1] William H. Welch and Simon Flexner. Johns Hopkins Hosp. Bull., Balt., 1892, III, 17-18.

[2] The Pathology of Toxalbumin Intoxication, by Simon Flexner. Johns Hopkins Hosp. Rep., Balt., 1897, VI, 1-409.

There was a considerable excess of clear fluid in the peritoneal cavity. Both layers of the peritoneum were reddened, the vessels of the visceral layer being especially injected. The spleen was enlarged to double the average size. It was mottled, and the white follicles were distinctly outlined against the red ground. The liver was dark in color and contained much blood. On the surface a prominent yellowish white area 2 mm. in diameter, surrounded by a zone of hyperaemia, was observed. Smaller dot-like points of the same color and general appearance were seen elsewhere in the liver. The kidneys were congested and the cut surface was cloudy. The adrenal glands appeared normal, as did the mesenteric glands.

The pleural cavity did not contain such a marked excess of fluid. The pericardial sac, however, was distended with clear serum. Under the epicardium were many ecchymotic spots. The lungs exhibited areas of intense congestion, or actual haemorrhage of the tissues. The glands of the thorax were, perhaps, swollen.

The examination of frozen sections showed the heart muscle to be slightly fatty. The epithelium of the tubules of the kidney was extremely granular and much swollen, but not fatty. The liver was very fatty; the lighter areas and dots were seen to correspond to foci of dead liver cells, whose refraction was much greater than that of the normal cells.

Cultures were made from the blood and organs of the animal and they remained sterile. Cover-slips were also examined and no organisms found.

The histological lesions observed in this case are identical with those described by us in connection with the inoculation of the living organisms. Lymphatic apparatus: In general, the changes are the same throughout. They are found in the greatest intensity in the glands of the axillary and inguinal regions, and less in the bronchial, cervical, mediastinal and mesenteric glands. Yet these are considerably affected. The same fragmentation of nuclei, affecting the lymph nodes and sinuses is met with. These fragments exhibit the variety of form previously described by us, and they have the same affinity for coloring agents. Much of the nuclear detritus is free, but a part is contained within large pale cells. In the spleen there is a similar diffuse fragmentation of the nuclei of the spleen cells. Both the lymphoid cells of the follicles and the larger cells of the sinuses are affected. Like the lymphatic glands, some of the nuclear detritus is enclosed in large cells. Besides the destruction of cells in the spleen there is haemorrhage into the organ, or an extreme degree of congestion, so that the tissue elements are widely separated from one another. Nuclear figures occur in the lymph glands and spleen. In the former they are found among the fragmented cells.

Stained sections of the liver, especially those stained in methylene blue and eosine, show the yellowish white areas to be composed of hyaline, necrotic liver cells. The necrotic cells stain deeply in the eosine, and they are usually devoid of nuclei. They form, on the whole, more or less definite foci of hyaline cells into which the leucocytes have wandered. The largest area was 2 mm. in diameter, and the outlines of it were formed by haemorrhage into the tissues, corresponding with the hyperaemic zone spoken of above. The cells in this focus have lost their nuclei and they are intensely refractive. Many of the dead cells have retained their individuality, and indeed, their borders are more distinct than those of the normal cells. Others, however, tend to become fused together and to lose their individual cell outlines. Occasionally, outside the main focus of hyaline cells, single necrotic cells occur which are surrounded by quite normal ones. Many leucocytes have wandered into this area of dead cells, and they are especially abundant at one place in the focus in which the hyaline and necrotic cells are in process of disintegration. An exquisite nuclear fragmentation is to be observed throughout this area.

Should the focus just described be compared to many similar foci which occur in the liver of animals dead of inoculation with the bacilli themselves, it will be seen to contain more leucocytes (polymorphonuclear) within it. The explanation of this fact would seem to depend somewhat on the incubation time, but more on the progression or stage of the necrotic process. Inoculation of the bacilli usually leads to death in a very short time, often in 24 to 48 hours. In this inoculation with the toxic products alone, the incubation period exceeded three weeks. On account of this, time has been allowed for the softening and disintegration of the dead cells, and leucocytes have been strongly attracted to these foci.

In the kidneys, besides the condition described in the frozen sections, a slight fragmentation of the nuclei of the epithelium of the tubules is encountered. The lungs exhibit areas of haemorrhage into the alveoli. and in many of these there has been a desquamation of the alveolar epithelium. Sometimes the desquamated epithelial cells are quite normal in appearance, while at others they have fragmented nuclei. The collections of lymphoid cells around the medium-sized and larger bronchi show, however, more cells, the nuclei of which have suffered in this way.

The blood vessels of the tissues generally contain fewer leucocytes in this instance than in those cases in which the bacilli were introduced beneath the skin. By the latter method an intense local inflammatory process is provoked, associated with the emigration of large numbers of polymorphonuclear leucocytes. In the former, in which the filtrate, free from organisms, is used for inoculations, the local process is reduced to nil, there is no emigration of leucocytes, and the disease is general from its inception. This difference is sufficient to account for the occurrence of leucocytes in the one case and its absence in the other.

It may be considered as established now that the toxic products and not the bacilli themselves invade the tissues in diphtheria. This fact would at once suggest that the general lesions (those produced at a distance from the seat of inoculation in animals, and the situation of the local process in human beings) were the effects of the soluble poison diffused through the body. Hence, it was desirable to demonstrate this assumption experimentally; and it is not unimportant to know that the lesions in the tissues produced by the bacilli and the toxic principal on the one hand, and the toxic principle alone on the other, are in perfect correspondence with each other. And, moreover, it would seem not to be superfluous to emphasize the occurrence of definite focal lesions in the tissues of the body, produced by a soluble poison circulating in the blood.

BACTERIOLOGICAL INVESTIGATIONS OF DIPHTHERIA IN THE UNITED STATES[1]

It is intended in this report to present the more important results of the bacteriological study of diphtheria by American investigators, so far as these results are believed to be of interest for the purposes of this Congress.

The most extensive bacteriological researches on diphtheria in this country have been made by Prudden and Park in the Pathological and Bacteriological Laboratories of the College of Physicians and Surgeons, Columbia College, New York (Director, Dr. T. M. Prudden), and by Park (partly in cooperation with Beebe) under the Division of Pathology, Bacteriology, and Disinfection, Health Department of New York (chief of this Division, Dr. Hermann M. Biggs); by Welch, Abbott, Booker, and Flexner in the Pathological Laboratory of the Johns Hopkins University and Hospital (Director, Dr. W. H. Welch); by Koplik, working in the Carnegia Laboratory, Bellevue Hospital Medical College (under the direction of Dr. E. K. Dunham); by Abbott, Ghriskey and Ravenel in the Laboratory of Hygiene, University of Pennsylvania (under the direction of Drs. J. S. Billings and A. C. Abbott); by Councilman, Williams, Morse, Townsend, Wright and Emerson in Boston, chiefly in the Boston City Hospital, and in the Pathological Laboratory of the Harvard Medical School (Director, Dr. W. T. Councilman). The references to articles are given at the end of this paper.

The writer is indebted for original abstracts of their work (some if it hitherto unpublished) and their preparation for this report, to Drs. Prudden, Park, Koplik, Abbott, Ravenel, Booker, Morse, Wright, and Emerson; and he desires to express to these gentlemen his cordial thanks for their kind cooperation, and also to Dr. Councilman for procuring the abstracts of work done under his supervision. It was intended at first to publish *seriatim* these abstracts, but it is believed that a systematic presentation

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[1] A report in behalf of the American Committee on Diphtheria to the Eighth International Congress of Hygiene and Demography, held in Budapest, September 1 to 9, 1894. This committee, of which Dr. John S. Billings is Chairman, was appointed by the Secretary General of the Eighth International Congress of Hygiene and Demography, together with similar committees in other countries, to report to the Congress upon various questions relating to diphtheria. To the writer was assigned the subject of this report.

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of the combined results under suitable headings will be more useful. The purposes and limits of this report permit the statement of only some of the results of American workers, and these often in much abbreviated form. The writer, whose part is only that of a referee, will make free use, often without further acknowledgment, of the words of the authors. The larger part of the investigations of Park reported in this paper are unpublished. This report embraces the results up to May, 1894.

In accordance with the programme of the Congress, especial attention will be given in this report to the results thus far obtained in the United States as to the following points:

The application and value of bacteriological methods in the diagnosis of diphtheria; the proportion of cases of pseudomembranous inflammations of the throat and air passages due to the Loeffler bacillus; the various localizations of the diphtheria bacillus; atypical forms of diphtheria, and false diphtheria; the persistence of the Loeffler bacillus in the throat after recovery from diphtheria; the occurrence of this bacillus outside of persons affected with diphtheria; the characters of pseudodiphtheria bacilli.

Historical.—The first bacteriological study of pseudomembranous inflammations of the throat by modern methods in this country was by Prudden in 1889. He studied by microscopical examination and cultures twenty-four cases diagnosticated as diphtheria in young children, most of them in New York hospitals in which the affection had assumed the character of an epidemic. In a few of these cases the pseudomembranous inflammation occurred by itself, but in most of them it was associated with scarlatina, measles, or suppuration. In all but two of these cases he found a streptococcus, usually in large numbers in the local lesions and in small numbers in a few cases in the viscera. Loeffler's bacillus was not found in any of this group of cases.

Prudden has withdrawn the name *Streptococcus diphtheriae,* originally applied by him to the streptococcus found in these cases, which did not appear to differ from *Streptococcus pyogenes.* It is clear that the group of cases first studied by Prudden were not cases of genuine diphtheria, but were for the most part cases of secondary pseudomembranous inflammations, which are now known to be oftener due to a streptococcus than to the Loeffler bacillus.

In February and March, 1891, Welch and Abbott published the results of the bacteriological examination of eight cases of primary diphtheria, in all of which the Loeffler bacillus was found.

In April, 1891, Prudden published the results in twelve cases of diphtheria unassociated with complicating lesions, and found the bacillus of Loeffler in all but one.

Since these first publications the number of cases of suspected or established diphtheria which have been examined bacteriologically in the United States, so as to be available for statistical analysis, has been between 6000 and 7000 (May, 1894).

Several hundred of these cases were examined in hospitals, dispensaries, and private practice, but by far the larger number were examined under the Health Department of the City of New York. Under the intelligent and efficient direction of Dr. Biggs the Health Department has entered upon an undertaking which, upon such a scale, is believed to be hitherto unparalleled, to wit: the systematic examination of all cases of suspected diphtheria (unless objection is made by the attending physician or except certain cases in which it is not deemed advisable to disturb the patient) occurring in New York—the study of the cultures thus obtained being made by Dr. William H. Park and Mr. Alfred Beebe, who are thoroughly trained and careful bacteriologists. Some of the results of this undertaking will be considered in this paper.

All the bacteriological aspects of diphtheria and other pseudomembranous inflammations of the throat, with the exception of systematic study of immunization and serum-therapy, have been investigated by the various American workers already mentioned. Attention, however, will not be given in this paper to the purely morphological and cultural characters of the diphtheria bacillus, except as they relate to the question of differentiation of the pseudodiphtheria bacillus.

Technique.—In general the methods of examination have been those usually employed, and only a few points need to be especially mentioned.

To obtain the material for cover-slips and culture from the suspected throat, Park uses sterile cotton swabs made by wrapping firmly a small pledget of absorbent cotton around the roughened end of a thin, stiff steel rod six inches in length. These swabs placed in an equal number of glass tubes, plugged with cotton, are sterilized for one hour by dry heat at 150° C. A similar method is employed by Councilman and others in Boston. Koplik prefers, both to the cotton swab and to the ordinary platinum wire, especially for removing tonsillar plugs, a small sterilized thin scoop in the form of a long bent probe.

All are agreed as to the superiority of Loeffler's blood serum mixture for making original cultures from the throat. The blood serum mixture is now generally prepared by direct sterilization and solidification in the steam or hot air sterilizer at a temperature a little below 100° C., instead of the old method of fractional sterilization at a lower temperature. The loss of transparency in the medium is compensated by the greater facility in preparation, and no difficulty is found in seeing the colonies after twelve to eighteen hours. Egg-albumen, as recommended by Johnston, and urine agar were found by Park less satisfactory than blood serum.

While recognizing that the bacillus of diphtheria grows well on agar when planted from cultures on blood serum or other media, and that the agar colonies are the most characteristic and best adapted for microscopical study, Park calls attention to the uncertainty of glycerin agar as a medium for making original cultures from the exudate. This uncertainty is due not only to unavoidable variations in the composition of the beef broth, and to the less luxuriant character of the growth of *Bacillus diphtheriae* as compared with its coincident growth with other bacteria upon blood serum, but also to the observation that when *Bacilli diphtheriae* are few in number or have been affected by previous application of antiseptics, they may fail to grow upon agar when blood serum inoculated at the same time and with the same exudate presents growth of the bacilli. Of course, the use of antiseptics shortly before making the inoculation of the tube is likely to render the cultures useless.

When it is desired to determine accurately the kinds of bacteria associated in the exudate with the diphtheria bacillus, especially the presence of streptococci, it is desirable not to rely upon blood serum alone, but to make also agar plates from the exudate.

In testing the virulence of diphtheria cultures emphasis is laid, in all of the later work, upon observing precautions which have been noted especially by Behring and by Escherich. Thus Koplik selects young guinea pigs weighing from 350 to 450 grammes, and injects 0.1 to 0.5 c. c. of a bouillon culture forty-eight hours old in the thermostat. The quantity injected should be taken from the full culture after shaking and not after pouring off the supernatant fluid from the deposit. Park also emphasizes the importance of selecting, if possible, young guinea pigs and injecting $\frac{1}{4}$ to $\frac{1}{2}$ per cent, in special cases even 1 per cent, of the body weight of a forty-eight hour bouillon culture grown at 37° C.

For ordinary diagnostic purposes cover-slip preparations and cultures are considered sufficient without awaiting the result of the animal experiment. In all of the cases both cover-slip preparations and cultures were made, and in many also inoculation of guinea pigs.

Methods Adopted by the Health Department in New York.—Under the Division of Pathology, Bacteriology, and Disinfection, of which the Director of the Bacteriological Laboratory is Dr. Hermann M. Biggs, and the inspector of Diphtheria Dr. William H. Park, the Health Department of New York has organized a system which renders available to physicians throughout the city the bacteriological diagnosis of diphtheria, and which utilized in the interests of preventive medicine the important discoveries relating to the causation and mode of spread of diphtheria. These efforts have met, for the most part, with hearty cooperation on the part of physicians.

For carrying out this and similar work, a bacteriological laboratory has been equipped and competent bacteriologists and a force of physicians as inspectors are employed. Cultures are made, not only when specifically requested by physicians, but also when no objection is raised by the attending physicians, diphtheria being, of course, a notifiable disease. Especial attention is given to examinations in boarding houses, hotels, and tenement houses.

In general, cultures are not made for diagnosis from secondary cases in families where the first case was proven bacteriologically to be diphtheria, or in cases of croup in which tracheotomy or intubation has been performed, the knowledge to be gained by cultures in such cases being too slight to compensate for the disturbance of the patient. All such cases, and, in fact, all suspected cases in which cultures are not obtained, are regarded as diphtheria. Later cultures are, however, made to determine when the bacilli have disappeared and the premises are ready for disinfection.

Indeed, especial importance is attached to these later cultures, for in view of an extensive research to determine the length of time during which diphtheria bacilli may persist in the throat, the Health Department has adopted the rule, " That no persons who has suffered from diphtheria shall be considered free from contagion until it has been shown by bacteriological examination, made after the disappearance of the membrane from the throat, that the throat secretions no longer contain the diphtheria bacilli, and that until such examinations have shown such absence all cases in boarding houses, hotels, and tenement houses must remain isolated and under observation. Disinfection of the premises, therefore, will not be performed by the department until examination has shown the absence of the organisms." Cultures are made also from the apparently healthy throats in an infected household for reasons which will appear later in this report.

The culture tubes may be inoculated by the attending physician, if he so desires; otherwise the cultures are made by the inspector of the district in which the case occurs. The outfits for making the cultures, consisting of a box containing a tube of blood serum and a sterilized swab in a test tube, are distributed to about forty stations at convenient points throughout the city, these being mostly druggist shops. A list of these stations is published, at which physicians can obtain the outfit free of cost.

Printed directions for making the cultures in suspected cases of diphtheria are supplied with the outfit, and are as follows:

" The patient should be placed in a good light, and, if a child, properly held. In cases where it is possible to get a good view of the throat, depress the tongue and rub the cotton swab gently, but freely, against any visible exudate.

"In other cases, including those in which the exudate is confined to the larynx, avoiding the tongue, pass the swab far back, and rub it freely against the mucous membrane of the pharynx and tonsils. Without laying the swab down, withdraw the cotton plug from the culture tube, insert the swab, and rub that portion of it which has touched the exudate gently but thoroughly all over the surface of the blood serum. Do not push the swab into the blood serum, nor break the surface in any way. Then replace the swab in its own tube, plug both tubes, put them in the box, and return the culture outfit at once to the station from which it was obtained.

"A report will be forwarded the following morning by mail, or can be obtained by telephone, after 12 noon."

There follows a list of the stations from which the culture-outfits can be obtained free of cost.

The inoculated tubes and swabs are sent from the stations to collecting depots, whence they are taken by the collectors on the same day to the laboratory. There is filled out and returned with the cultures a printed form giving the name and address of the attending physician and of the maker of the culture, the date, the name, age, and address of the patient, the clinical diagnosis, various details relating to the supposed causation, the duration and character of the disease, the use of antiseptic applications, and a statement as to whether the inoculation was satisfactory. Another printed form is to be filled out for later cultures.

From time to time explanatory circulars as to the character and results of the work and the rules adopted by the department are issued to physicians.

There are printed forms to be filled out in reporting to physicians the results of the cultures; one form is for cases in which diphtheria bacilli are found; another is for cases in which the bacilli once present have disappeared, and on this is the statement that the case is ready for disinfection, if other circumstances allow; a third form is for cases in which the cultures do not show the presence of diphtheria bacilli; and a fourth in the case of cultures which do not permit an exact bacteriological diagnosis with a statement of reasons for the failure, and, if need be, another culture is requested.

Specimens of these various printed forms accompany this paper for the inspection of those attending the Congress.

The results thus far obtained by this system have been satisfactory. From May 4, 1893, to May 4, 1894, 5611 cases of suspected diphtheria were subjected to bacteriological examination, some of the results of which will be stated later in this report.

As to the reliance to be placed upon a culture in making a diagnosis, Park states, as the result of the very large experience accumulated by the Health Department:

"The examination by a competent bacteriologist of the bacterial growth in a blood serum tube which has been properly inoculated and kept for fourteen hours at the body temperature can be thoroughly relied upon in cases where there is visible membrane in the throat, if the culture is made during the period in which the membrane is forming, and no antiseptic, especially no mercurial solution, has lately been applied. In cases in which the disease is confined to the larynx or bronchi, surprisingly accurate results can be obtained from culture, but in a certain proportion of cases no diphtheria bacilli will be found in the first culture, and yet will be abundantly present in later cultures. We believe, therefore, that absolute reliance for a diagnosis cannot be placed upon a single culture from the pharynx in purely laryngeal cases. The apparent mistakes, however, have been few. Cultures cannot be certainly relied upon after the membrane begins to disappear."

The great majority of physicians desire cultures to be made and appreciate the value of an accurate diagnosis. Cases which are proved not to be diphtheria are not required to be isolated and are not kept under supervision by the department; until such proof, however, suspicious cases are treated as diphtheria.

The duration of the period of isolation and the time for disinfection are determined by the bacteriological examination in accordance with the rule already quoted. The increased value of statistics as to the distribution, prevalence, mode of spread, clinical characters, mortality, and treatment of diphtheria when the diagnosis is based upon a bacteriological examination, requires no especial emphasis. Some of the scientific results which have been obtained by Dr. Park and Mr. Beebe from this immense material will be mentioned later in this article.

Proportion of Cases of Suspected Diphtheria Proved by Bacteriological Examination to be True Diphtheria.—There are several points to be considered in estimating the value of statistics upon this point. The character of the cases selected for examination, whether wholly characteristic or less characteristic of diphtheria, will be of decisive influence. The degree of suspicion in a given case may be very slight, or one physician may consider the case highly suspicious or certain, and another may regard it as very doubtful or certainly not diphtheria.

It is particularly in the doubtful cases that a bacteriological examination is desired and is of the most importance. Diphtheria may occur without any false membrane and as an angina or tonsillitis of very mild character.

In statistics based upon the statements of a large number of physicians as to the character of a local exudate it doubtless may happen that simple pultaceous and purulent exudates may be mistaken for genuine fibrinous exudates. The inclusion of cases of pseudomembranous angina, occurring in scarlet fever or other diseases, or even during the prevalence of scarlet fever, will influence the statistical result. Special local conditions, such as the selection of cases exclusively from children's hospitals, and probably the time of year, may likewise have an influence upon the result. The care with which the cultures and cover-slip preparations are made and examined, the period of the disease at which they are made, the previous local application of antiseptics, are factors which will influence the result.

These considerations (and still others might be mentioned) may render the statistics of different observers not strictly comparable, and they indicate that some statistics are likely to be more accurate and valuable than others.

We owe to Park and to Morse the most extensive statistics as to the subject now under consideration. In his first published series Park examined 140 pseudomembranous cases uncomplicated with scarlet fever, in which the clinical diagnosis of probable diphtheria had been made. He found the Loeffler bacillus in 54 cases (39 per cent). Of the 86 remaining cases the membrane was confined to the tonsils in 58, and these latter were all mild.

In his second published series, 104 uncomplicated cases were examined and the Loeffler bacillus was found in 73 (70 per cent). Dr. Park has kindly furnished the writer his later, as yet unpublished, statistics (partly in cooperation with Beebe), which will be included in a report to the Board of Health of New York. In this third series, 5611 cases of suspected diphtheria occurring in New York during the year ending May 4, 1894, were examined bacteriologically. A few of these cases complicated scarlet fever, but the precise number of such cases is not stated. The Loeffler bacillus was found in 3255 cases (58 per cent), and was absent in 1540 cases (27 per cent). In 816 cases, although no diphtheria bacilli were found in the cultures, yet either because the cultures were made after the fourth day of the disease, or because the exudate was imperfectly obtained from the throat, or because the culture media had become contaminated or were too dry, the cases were considered to be of a doubtful nature as far as the bacteriological examination was concerned. If the doubtful cases be thrown out, which, however, it is hardly fair to do, as most of them are considered by Park not to have been diphtheria, we have, in 4795 cases of suspected diphtheria, about 68 per cent of cases of true diphtheria, and 32 per cent which were not diphtheria.

Morse found, in 301 cases without scarlet fever admitted to the diphtheria ward of the Boston City Hospital, the Loeffler bacillus in 217 (72 per cent).

If we add all of the cases of suspected diphtheria in Park's and Morse's statistics we have 6156 cases, of which 3599, or 58.5 per cent, were proved bacteriologically to be true diphtheria. If we reject from Park's third series the cases in which the bacteriological examination was unsatisfactory we have 5340 cases, of which 67.5 per cent were cases of true diphtheria.

Park's first series was remarkable on account of the small percentage of cases of true diphtheria. The examinations were made in February and March, and scarlet fever was very prevalent, diphtheria less so. His experience at this time he considers to have been exceptional, and it has not been repeated. His own conclusion is, from his later observations, that about 60 per cent of the cases of suspected diphtheria in New York are true, and about 40 per cent are false, diphtheria.

We are, of course, not to infer that only 60 per cent of the cases which are considered by physicians to be undoubted diphtheria are really diphtheria, but that this is about the percentage of cases of genuine diphtheria in the pseudomembranous inflammations of the throat in which there is more or less suspicion of diphtheria.

Upon each blank sent with a culture tube by the Health Department of New York, a space is left for the voluntary recording of the clinical diagnosis by the physician. In 1210 cases of true diphtheria the physician's diagnosis was recorded. In 1011 the case was considered on clinical grounds to be diphtheria, in 102 to be doubtful, and in 87 to be probably false diphtheria. In 675 cases in which the diphtheria bacilli were absent a diagnosis of diphtheria was made in 50, the rest being all considered to be in doubt.

In this connection Park says: "The cases which present the characteristic appearances of well developed diphtheria regularly contain in the pseudomembranes abundant diphtheria bacilli. It is the slightly developed cases in which a culture is most needed. Here without cultures we need to go more by the history of exposure than the appearance of the case. In a family where an undoubted case of diphtheria existed, any form of sore throat, with or without exudate, in a child or adult, would be probably a case of diphtheria, while a similar appearing tonsillitis or pharyngitis in a person who had not been exposed to diphtheria would be, in all probability, a case of ordinary non diphtheritic throat inflammation." In only about one-half of the cases of diphtheria examined by the Health Department of New York did careful inquiry reveal any connection with other diphtheria cases.

The statistics which have been cited are based upon the examination of pseudomembranous inflammations of the throat and air passages mostly uncomplicated with scarlet fever.

Of particular significance is the outcome of Morse's study: that in no less than 28 per cent of the cases admitted to the diphtheria ward of the Boston City Hospital, diphtheria bacilli were not found.

Membranous Croup.—Welch and Abbott, Booker, Williams, and others have reported cases of diphtheria in which the pseudomembrane was confined to the larynx and lower air passages. In Park's statistics there are 286 cases (283 children and 3 adults) in which the membrane was entirely or chiefly confined to the larynx or bronchi. In the cultures from 229 of these the Loeffler bacillus was found, and 167 of these 229 cases showed no pseudomembrane or exudate above the larynx, while in the remaining 62, although the larynx was mainly involved, there was also some membrane or exudate present on the tonsils or pharynx. In the remaining 57 no diphtheria bacilli were found, but of these, the cultures in 17 were unsatisfactory, leaving the bacteriological diagnosis doubtful. In the 40 cases in which the diphtheria bacilli were absent the disease was confined to the larynx or bronchi in 27, while more or less exudate or membrane was present on the tonsils or pharynx in 13. Of the cases of membranous croup in New York, therefore, at least 80 per cent are diphtheria and only 14 per cent were undoubtedly not diphtheritic. Cases of membranous croup were observed to be the origin of cases of characteristic pharyngeal diphtheria.

The comparatively small number of laryngeal cases examined is accounted for by the custom of the department inspectors not to make cultures in cases which have been intubated or which seem so sick that the family might think injury had been done by inserting the swab.

In a personal communication Dr. Park writes that he has no doubt as to the occurrence of nondiphtheritic pseudomembranous laryngitis. He speaks of two cases in which the microscopical examinations and cultures made both during life and after death showed no diphtheria bacilli, but streptococci were present. Booker has reported such a case complicating measles.

Fibrinous Rhinitis; Nasal Diphtheria.—Park reports 10 cases of fibrinous rhinitis in all of which the Loeffler bacillus was found. All were typical and of the usual benign character. In 9 the membrane was confined to the nose, and in one there was also slight fibrinous exudate on the tonsils. Only the 6 cases in his first series are reported in detail. In 4 the Loeffler bacillus was associated with streptococci and in 2 with staphylococci. The virulence of the diphtheria bacillus was tested in 5 cases. The bacillus from one case killed a guinea pig in four days; those from two cases in five days, and those from two other cases rendered the guinea pigs sick, but the animals eventually recovered. In addition to this weakening of virulence Park found that the bacilli from the first six cases grew more feebly than usual both on blood serum and agar, and died out in cultures sooner than usual.

Abbott has published 3 cases of fibrinous rhinitis, and Ravenel, working under Abbott's direction, has kindly sent me an account of 2 additional cases which we will publish. All of these 5 cases were of benign character, the membrane being confined to the nasal cavity. Two were in sisters in the same family. In all the Loeffler bacillus was present in the membrane; in 3 it was of the customary virulence, and in 2 it produced only local reaction, in large doses failing to kill even half-grown guinea pigs.

Townsend observed in 14 cases of diphtheria, mostly of mild character, occurring in the Children's Hospital in Boston, 7 cases in which the nose was affected. Of the latter, in 5 cases the disease was primarily nasal, the membrane being confined to the nose in 4. These 4 cases of primary and exclusively nasal diphtheria were mild. In all the Loeffler bacillus of customary virulence was found. He emphasizes the case with which these primary nasal cases may be overlooked if the membrane remains confined to the nose, the importance of a bacteriological examination in all suspicious cases of nasal discharge, the mild character of many of the cases, their resemblance by superficial examination in some cases to an ordinary coryza, and the danger of such cases as sources of infection.

Park mentions an instance in which a child with only a slight nasal discharge, in which Loeffler bacilli were demonstrated, gave rise to diphtheria in four children, of whom two died. The first child came from a family in which there had been a case of diphtheria three weeks before.

Clinically Atypical Diphtheria.—The most important bacteriological study of this subject has been made by Koplik, who has kindly furnished the following abstract of his work:

"The writer has for the past three years directed his attention chiefly to the study of those forms of diphtheria which clinically do not present any of the classical local symptoms of the disease. The direct outcome of these studies has been to demonstrate how multiform diphtheria may be, from a clinical standpoint, in its various local manifestations. The first series of studies was published in the 'New York Medical Journal,' August 27, 1892, and 'Archives of Pediatrics,' September, 1892. In these papers the writer gave the results of studies of the following forms of diphtheria:

"*A*. Cases in which there is from the onset and through the whole course of the disease no local manifestation of membrane, either *diffuse* or *punctate*. These cases may be so exceedingly mild in their course as to simulate a simple catarrhal angina, or they may be accompanied not only by anginal symptoms, but laryngeal symptoms, such as croupy cough and croupy breathing. In the latter case, early in the disease, we find upon the most careful inspection of the fauces no signs of membrane; later, this picture may persist, or there may appear punctate areas of membrane upon the tonsils or pillars of the fauces. The point of greatest moment, however, is that at first and for days the closest inspection of the fauces and epiglottis reveals no signs of membrane. The croupy cough and breathing reveals involvement of the larynx. Where only the cough is manifest it is to be presumed that in the larynx the condition is much like that found in the throat, an intense inflammation without membrane.

"It is, of course, impossible to establish the exact anatomical conditions. In these cases I have fixed the diagnosis of diphtheria by scrapings from the tonsil and fauces. The most dangerous of these cases in spreading infection are those which present symptoms of a mild catarrhal angina. The writer has published a series of cases occurring in the same family of children who presented these mild anginal symptoms. They communicated their anginal diphtheria to one another. Some of the cases developed membrane (punctate, in other cases diffuse of fauces and larynx), resulting in fatal laryngeal stenosis. On the other hand, the writer has published a series of cases of children who presented all symptoms of acute catarrhal angina, with signs of lacunar amygdalitis. In these cases the croupy cough and breathing were not present. These were not diphtheritic. The only test is the bacteriological one.

"*B.* A class of cases in which the tonsils are covered by a pultaceous mass of exudate, and no consistent membrane. These cases may or may not be true diphtheria.

"*C.* A large class of cases presenting punctate form of membrane; some of these spots of membrane may be isolated and minute on the tonsil; in some forms the specks of membrane are depressed in the surface of the tonsil. In other forms minute or larger ulcerations exist. These cases may be diphtheritic, and in the vast majority of cases are true diphtheria. In other cases we fail to find the Loeffler bacillus.

"*D.* The most important class of clinically atypical diphtherias are those cases which begin or even run their entire course with the clinical local picture of a typical lacunar or follicular amygdalitis. In my last paper, entitled 'Acute Lacunar Diphtheria of the Tonsils' (New York Medical Journal,' March 10, 1894) these cases are divided into groups. In the first group of cases the course of the disease is that of an exceedingly mild lacunar or follicular amygdalitis. The fauces are injected, the tonsils are enlarged, there is an indication here and there of a lacuna, by a yellow spot; the lymphatic glands at the angle of the jaw may or may not be enlarged; the constitutional symptoms are almost *nil.* Extraction of the contents of the lacunae or follicles shows the presence of *Bacillus diphtheriae.*

In the second group of cases the tonsils are more inflamed, the lacunar plugs protrude from the tonsils in the form of soft fibrinous masses; the patients seem quite ill, as if suffering from a severe amygdalitis. In these cases also the lacunar plugs are found to contain the Klebs-Loeffler bacillus in almost pure culture. Here also glandular nodes are found at the angle of the jaw. The course of these cases clinically is interesting; some never develop membrane as a mass on the tonsils; others after the third day develop distinct thick confluent membrane on the tonsils. Others remain lacunar in the local tonsillar picture, but develop laryngeal diphtheria, with croupy cough and breathing. In this class the lymph nodes at the angle of the jaw may be much enlarged in packets, or not much above what is found in ordinary anginas. These cases may give rise to other cases in the same family in which the course of the disease is that of a simple lacunar amygdalitis, but which upon examination prove to be true diphtheria.

The third group of cases are those in which the disease begins with the picture of severe lacunar amygdalitis, but the prostration of the patient is exceedingly great; the temperature may be low. There is great glandular swelling at the angle of the jaw. After a few days the prostration is much greater; there appears nasal discharge, and small shreds of membrane are discharged from between the much swollen tonsils. The tonsils themselves are now covered with one continuous membrane, but here and there shreds are seen on the surface of the eroded tonsil. These cases are the septic lacunar diphtherias with nasal symptoms, and give a bad prognosis.

"In a series of 39 cases of acute lacunar or follicular inflammation of the tonsils, 12 were proven to contain the Loeffler bacilli in the depths of the tonsillar lacunae. Thus, fully one-third of the cases were diphtheria. It is not intended to draw any percentage conclusion from this, but it demonstrates how insidious an affection lacunar amygdalitis may be. ('New York Medical Journal,' March 10, 1894.)"

Diphtheria sine membrana has been observed also by several other American investigators. Reference has already been made to Park's statement that any form of sore throat in a person exposed to diphtheria is likely to be referable to the diphtheria bacillus. He says: "Under diphtheria should be included all inflammations of mucous membranes due to the diphtheria bacillus of Loeffler. A case with an acute hyperaemia of the mucous membrane in which the Loeffler bacilli are present is considered as truly a diphtheria as one with pseudomembrane or exudate." Only the bacteriological examination can determine the true character of these cases. It is to be borne in mind, as pointed out by Councilman, that the absence of the pseudomembrane in the places where it is ordinarily found is not proof that it is not present elsewhere, perhaps in some place not accessible to examination.

Other Localizations of the Diphtheria Bacillus and its Distribution at Autopsies.—Councilman has found the diphtheria bacillus in one case of otitis media following diphtheria and in two cases of otitis media, without affection of the throat, in measles. Townsend observed a case in which an old tracheotomy wound was attacked and the disease spread to the bronchi, proving rapidly fatal, with demonstration of the Loeffler bacillus. Park found diphtheria bacilli in two cases in wounds of the finger received by physicians in intubating children with diphtheria. In one case the bacilli persisted in the wound for six weeks. Wright (unpublished results communicated to the writer) has demonstrated the presence of this bacillus in excoriated or ulcerated surfaces of the skin in 7 cases, in paronychia in 1, in mastoid abscess in 1, in purulent conjunctivitis in 1—all in cases of diphtheria. At autopsy he cultivated the bacillus from the ecchymotic mucous membrane of the stomach in 2 cases, and from oedematous tissue behind the oesophagus in 1. Wright also reports that in 10 out of 14 autopsies in cases of diphtheria bronchopneumonia was present, and that in 9 cases the Loefller bacillus was found in the lung, and in the remaining case only the streptococcus. In 7 of the 9 pneumonias streptococci accompanied the Loefller bacillus, as well as in two other lungs in which no pneumonia existed. In 3 or 4 pneumonias *Diplococcus pneumoniae* was also present. *Staphylococcus pyogenes aureus* was also met in 4 pneumonias, once with the Loefller bacillus and three times in company with all of the three bacteria just mentioned. Flexner has found the Loeffler bacillus in two bronchopneumonias complicating diphtheria, and by microscopical examination of section demonstrated Loeffler bacilli, mostly within leucocytes, in the bronchopneumonic exudate. In both cases *Diplococcus pneumoniae* was, however, the predominant organism.

Howard has reported a case of acute ulcerative endocarditis (without diphtheria) with infarctions in the spleen and kidneys, in which, both by cultures and microscopical examination, bacilli indistinguishable morphologically and in cultures from the diphtheria bacillus, but devoid of virulence, were found abundantly and in pure culture in the valvular vegetations, the spleen, and the kidneys.

As to the presence of the diphtheria bacillus in internal organs at autopsy Booker reports a case of diphtheritic laryngitis in which at autopsy Loefller bacilli were found in the blood, spleen, and lungs. Wright has found this bacillus out of 14 cases dead of diphtheria in the lung in 13 cases (9 with bronchopneumonia), in the liver in 3, in the spleen in 2, in the cervical or bronchial lymph glands in 5, in the kidney in 1, in the blood in 1, and in the mesenteric lymph glands in 2. In 7 of these cases there was streptococcus septicaemia and in 2 a similar polyinfection with *Staphylococcus pyogenes aureus.* Two of the autopsies showed no marked lesions of diphtheria in the throat or trachea, the nasal cavities not having been examined. Both yielded virulent Loeffler bacilli—in one from the cervical lymph glands and in the other from the pharynx. In both the bacillus was found in the air passages. The bacillus, with slight virulence, was found also in certain of the organs in 3 autopsies on cases apparently not dead of diphtheria.

In guinea pigs dead of experimental diphtheria, Wright found the diphtheria bacillus in the liver in 19 out of 155 cases, in the spleen in 15 out of 152 cases, in the heart's blood in 7 out of 153 cases, and in the kidney in 4 out of 151 cases.

Both in these as well as in the human cases the bacilli were found in the organs in but very small numbers, as a rule.

In three guinea pigs the bacillus has been recovered by Wright from the seat of inoculation thirty-one to thirty-eight days after the date of inoculation.

Abbott and Ghriskey have observed frequently after inoculation of diphtheria bacilli into the testicles, less frequently after subcutaneous inoculation, of guinea pigs, small nodules in the omentum composed mostly of polynuclear leucocytes and containing the bacilli in large number.

Welch and Flexner have described in detail the histological changes in experimental diphtheria, and have shown that these are characterized especially by widely distributed areas of cell death with nuclear fragmentation. They have demonstrated that the local fibrinous exudate may be caused by dead, as well as living, diphtheria bacilli, and that the other lesions are referable to toxic products of the bacillus.

Bacteria Associated with the Diphtheria Bacillus.—All of the investigators have found, as a rule, other bacteria associated with the Loeffler bacillus in the local exudates of diphtheria, although this bacillus is the only one which is constantly present. The most common associated microorganism is *Streptococcus pyogenes.* Other bacteria which have been found are the regular buccal bacteria, *Micrococcus lanceolatus, Staphylococcus albus* and *aureus, Bacillus coli communis* (Morse), unnamed cocci and unnamed bacilli. Among these bacilli may be specified a short oval, slightly pointed bacillus, growing in long chains, running parallel to each, forming grayish white colonies on agar not unlike those of the Loeffler bacillus. This bacillus was frequently encountered by Welch and Abbott. Morse describes a short, motile, liquefying bacillus and a non motile diplobacillus growing invisibly on potato and not growing on gelatin. The only associated bacteria which are believed to exert pathogenic action are *Streptococcus pyogenes, Staphylococcus pyogenes, Micrococcus lanceolatus,* and a coccus observed by Koplik which he considers to be identical with that described by Roux and Yersin and by Martin. Differences, mostly of a minor character, have been noted both in morphology and cultural characters between the different streptococci, but they are not considered sufficent to establish definitely different species.

It is regarded as established by all the investigators that the course and symptoms of diphtheria may be definitely influenced by these associated pathogenic bacteria, particularly by the most common of all, *Streptococcus pyogenes.* Cases of general streptococcus septicaemia accompanying diphtheria have been observed, especially by Councilman and his coworkers. Bronchopneumonias, suppurations of lymph glands and the so-called septic forms of diphtheria are attributed to these associated bacteria, especially to the streptococcus. It does not appear, however, either from the statistics of Park or of Morse, that the mortality is materially higher in cases of diphtheria in which the streptococcus is associated with the Loeffler bacillus than when the Loeffler bacillus is present without streptococci. In fact, the tables of Morse show a higher mortality when the Loeffler bacillus is associated with pathogenic staphylococci than with streptococci. Morse, however, does not attach decisive weight to this inference, as only in 25 cases did he attempt a thorough differential study of all associated bacteria. Such study will always show the presence of other bacteria. In these 25 cases he found the streptococcus in 19 cases (9 without other bacteria in culture, except the Loeffler bacillus, 6 with *Staphylococcus aureus* or *albus,* or both), and *Staphylococcus aureus* or *albus* in 10 cases (4 alone, except the Loeffler bacillus). The general mortality in these 25 cases was 56 per cent, the mortality in the cases with the Loeffler bacillus and streptococci alone, 33 per cent, with Loeffler bacillus and staphylococci alone, 66 per cent, and with Loeffler bacillus, streptococci, and staphylococci, 66 per cent. As Morse says: "Statistics compiled from so small a number of cases are of little value." A similar conclusion as to the greater harmfulness of staphylococci than of streptococci is reached by Morse, contrary to the generally accepted opinion, also from the analysis of his larger table of 400 cases (301 without scarlet fever); but he admits that the comparatively small number of cases in which the streptococcus was found associated with the Loeffler bacillus (191 cases) may have been due to failure to recognize the minute, slow-growing colonies of streptococci in the twenty-four-hour blood serum tubes, and the arrangement of the cocci on cover-glass specimens alone is not decisive as to the diagnosis of staphylococci, which were found in 295 cases. A minute and careful differentiation of the bacterial species was attempted by Morse in only 25 cases. We cannot, therefore, consider it established that staphylococci are more harmful than streptococci, especially as this inference is opposed to that of other investigators, and as Morse doubtless often overlooked the presence of streptococci.

Pseudomembranous Inflammations of the Throat in Scarlet Fever, Measles, and Typhoid Fever.—In an epidemic of scarlet fever in Baltimore at the time when the city was unusually free from diphtheria, one-half of the cases seen by Booker had pseudomembranous affection of the throat. He has reported 11 cases of pseudomembranous angina (2 fatal) complicating scarlet fever, and 1 case of similar angina without exanthem in a family three members of which had scarlatina. In all of these cases, as well as in 4 scarlatinal anginas without pseudomembrane, Booker found streptococci as the predominant organisms, and in none was the Loeffler bacillus present. In one case croupous conjunctivitis due to streptococci was associated with the pseudomembranous angina. *Staphylococcus aureus* was found in 11 cases without apparent influence on the severity of the case. No difference was observed between the early and the late pseudomembranous anginas as regards the bacteria present. He attributes the absence of true diphtheria from so large a number of cases of scarlatina as due to the freedom of the city from diphtheria at the time. Booker describes with much detail the morphological and biological characters of the streptococci found, and divides them into groups, but these details will not here be considered.

Park in his first series of 159 cases reports 19 cases of pseudomembranous inflammation of the throat complicating scarlatina. In 17 streptococci predominated, and in only 2 were Loeffler bacilli present, these latter presenting diphtheritic laryngitis. Staphylococci were met in only a few cases.

The experience in Boston, as reported by Williams and by Morse, has been exceptional in the large proportion of cases of true diphtheria complicating scarlet fever. Of 97 cases of scarlet fever in the Boston City Hospital, of which 35 came from two homes for children, reported by Williams, membranous throats were present in 35. Of these 12 were due to the Loeffler bacillus, and in 23 the Loeffler bacillus was not found. In the 23 pseudodiphtheria cases the membrane was limited to the tonsils in 5 only, and in 3 it extended to the larynx. Of the 62 cases of scarlet fever alone, 8 died (13 per cent), of the cases of scarlet fever with diphtheria 6 died (50 per cent), of the 23 cases of scarlet fever with pseudodiphtheria 5 died (22 per cent). In a previous series of 86 cases of scarlet fever, of which 19 were complicated with membranous throats, but no bacteriological examination was made, the mortality in the latter group was 47 per cent, while in the 67 cases without membranous throats only 4½ per cent died.

In Morse's paper are probably included some of the cases reported by Williams, as the observations of both are from the Boston City Hospital. Morse reports 99 cases of pseudomembranous inflammation of the throat complicating scarlet fever. Of these 26 per cent died. The Loeffler bacillus was found in 23, with a mortality of 43 per cent, and was not found in 76, with a mortality of 21 per cent. In the pseudodiphtheritic cases streptococci were present in 61, staphylococci (in 12 alone and in 57 with streptococci) in 69, and other bacteria in 3 cases. Mention has already been made of the large number of staphylococcus cases in general in Morse's statistics.

It is the opinion of Morse, and of Councilman, under whose direction Morse worked, that the large proportion of cases of combined scarlatina and diphtheria was due to contagion in the hospital where the conditions were unfavorable for proper isolation. These unfavorable conditions will be remedied in the new hospital for infectious diseases now building. A few of the mixed cases, however, were admitted with double infection as shown by bacteriological examination at entrance. Most of the cases came from tenement houses. In Williams' cases the fact that 35 came from two public institutions may explain in part the large number of associated infections.

In contrast with these results in Boston it is interesting to note the observations of Booker, already cited, at a time and place in which diphtheria was notably absent. It is evident that the proportion of cases in which true diphtheria complicates scarlet fever depends largely upon local conditions, these relating to the prevalence of diphtheria and the opportunities for contagion.

Booker reports three cases of pseudomembranous inflammation accompanying measles. In all, streptococci and the *Staphylococcus aureus* were found, and in none the Loeffler bacillus. These cases occurred during the same period as the scarlatinal cases, when the city was exceptionally free from diphtheria. In two cases the membrane was limited to the larynx, there being no visible exudate. One of these was fatal, and at the autopsy the whole larynx wes covered by a tough, very loosely adherent, false membrane. Both microscopical examination and cultures of the membrane after death, as well as those made from the pharynx during life, failed to show Loeffler bacilli. This case, therefore, is a particularly conclusive one of the occurrence of membranous laryngitis without diphtheria. The third case presented pseudomembrane on the tonsils, pharynx and conjunctivae. Streptococci predominated with some colonies of *Staphylococcus aureus.*

In Park's first series of cases there is one case of laryngeal diphtheria accompanying measles. Loeffler bacilli were present. The child recovered after intubation. In another case, in which there was membranous laryngitis without diphtheria bacilli in the pharynx, measles developed on the eighth day. Intubation had been performed and was followed by recovery.

Williams reports one case of measles with diphtheria This case, however, may be the same one as appears in Morse's statistics, who reports four cases of measles with severe throat symptoms giving rise to the clinical diagnosis of diphtheria. One contained a few Loeffler bacilli and many staphylococci. Of these two died. Six cases had measles and scarlet fever together, but it does not appear that any of these had membranous throats. All recovered, and the Loeffler bacillus was not found in any. Streptococci were present in 5, staphylococci in 2, and *Diplococcus lanceolatus* in 2.

Of particular interest are four cases of typhoid fever complicated by diphtheria. reported by Morse. At least three of the cases developed diphtheria after admission to the hospital. One was a patient ill with typhoid fever in a private room. A nurse, several days after leaving the scarlet fever ward, had a sore throat, and was put in the same room over night. On the discovery of the Loeffler bacillus in her throat the next day, the nurse was transferred to the diphtheria ward. Four days later the patient with typhoid fever developed sore throat with membrane containing the Loeffler bacillus. The next day a typical scarlet fever rash developed, which was followed by desquamation. She ultimately recovered. One of the four patients died. At the autopsy were found lesions of typhoid fever with diphtheric pharyngitis, tonsillitis and laryngitis. Loeffler bacilli were demonstrated both *intra vitam* and *post mortem.* In addition, typhoid bacilli and general streptococcus infection were found in cultures after death. Wagner has already called attention to the greater frequency of pseudomembranous affections in typhoid fever when diphtheria is prevalent.

Pseudodiphtheria.—The name pseudodiphtheria is used by Park, Williams, and others to designate pseudomembranous inflammations of the throat and air passages not caused by the bacillus of diphtheria. The name is, for several reasons, objectionable, but no convenient substitute has been suggested. The term streptococcus diphtheritis fits most of the cases, but not all. The pseudodiphtheria bacillus is not concerned in the causation of pseudodiphtheria, or, if so, only in a very small number of cases.

We have just seen that the great majority of cases of pseudomembranous angina in scarlet fever are due to streptococci, although under special conditions the proportion of diphtheritic cases may be exceptionally large.

The statistics as to the proportion of cases of pseudomembranous inflammations of the throat not due to the diphtheria bacillus, exclusive of scarlatinal cases, occurring in New York and Boston, have already been given under the headings " Proportion of Suspected Diphtheria Proved to be Diphtheria," and " Membranous Croup." From these it appears that from about 28 to 40 per cent of the cases of nonscarlatinal pseudomembranous anginas are pseudodiphtheria, the percentage in some series being larger and in others smaller. The high percentage of such cases in Park's first series is altogether exceptional and probably due to special circumstances, among which Park mentions particularly the prevalence of scarlatina, the relative infrequency of true diphtheria, and the season of the year (February and March). Park writes that since then he has found the diphtheria bacillus in a series of cases in over 80 per cent.

Several foreign writers have been impressed with the large number of American cases of pseudomembranous angina and laryngitis in which diphtheria bacilli were not found, and have expressed some doubt as to the accuracy of the results. We attribute the striking differences in the reported statistical results of different investigators mainly to the class of cases selected for examination. If only typical and characteristic cases of diphtheria, be selected, as has been done by some observers, the proportion of cases in which the diphtheria bacillus is missed will be small, and may be *nil.* If, however, the less characteristic cases of diphtheria, concerning which, in many instances, no one can be sure without bacteriological examination whether they are genuine diphtheria or not, be included, there may be a relatively large percentage of cases of pseudodiphtheria. Foreign statistics, based upon the examination of cases admitted to the diphtheria wards of hospitals, in which cases, therefore, the clinical diagnosis is diphtheria, such as the statistics of Baginsky in Berlin, Martin in Paris, and Jansen in Switzerland, agree substantially with the New York and Boston statistics.

Koplik and Park describe with much detail the anatomical and clinical characters of the less characteristic diphtheritic and of the pseudodiphtheritic cases. Koplik's observations as to the former have already been cited. In these less characteristic cases neither the location nor the character of the diphtheritic inflammations of the throat suffice to distinguish positively the true from the false diphtherias, and a positive diagnosis must rest upon the bacteriological examination.

In the great majority of cases which have been examined with sufficient care, a streptococcus, not differing apparently from *Streptococcus pyogenes* or *erysipelatis,* has been the predominant organism in pseudodiphtheria and is believed to be the cause of this affection. In a small number of cases generally, but in Morse's examinations in a large number of cases, staphylococci appear to have been the causative microorganism. In a still smaller group of cases *Diplococcus lanceolatus* has been assigned as the causative factor. Koplik has met the coccus of Roux and Yersin. He also reports four cases of tonsillitis without membrane in which the pseudodiphtheria bacillus was found without the typical virulent diphtheria bacillus.

Park reports the mortality in 408 consecutive cases of pseudodiphtheria bacteriologically examined in New York, and not complicating scarlet fever, to have been only 1.7 per cent. Of the seven fatal cases only two were over five years of age. The five deaths in young children were all in laryngeal cases. He considers that nearly all of the deaths in pseudodiphtheria are due to complications, the most important being scarlet fever, membranous laryngitis, and bronchopneumonia. At the same period the mortality from true diphtheria in New York was 27 per cent.

In striking contrast to this low mortality in pseudodiphtheria is that found by Morse in the Boston City Hospital, where 25 per cent of the pseudomembranous cases without Loeffler bacilli and without scarlet fever died. The difference between Park's and Morse's results is probably to be explained by the fact that Park's cases were largely the usual mild ones in private practice, whereas Morse's cases were of sufficient severity to be sent to the hospital, many being laryngeal cases, and many dying within twenty-four hours after admission, the great majority being young children from crowded and insanitary tenement districts. The mortality in true diphtheria uncomplicated with scarlet fever was in Morse's cases 41 per cent. Park also has noted the greater mortality of pseudodiphtheria in the hospital cases as compared with those in private practice.

As is well known, there is much difference of opinion among physicians as to the contagiousness of throat affections due to streptococci and other cocci. In order to determine this point, the Health Department of New York, as reported by Park, investigated 113 consecutive cases of pseudodiphtheria occurring in 100 families, and at the same time 70 consecutive cases of diphtheria in 50 families. A history of contact with another case was found in 14 of the 113 cases of pseudodiphtheria, and in 33 of the 70 diphtheria cases. In 9 of the 100 families with pseudodiphtheria there was more than one case, and in 13 of the 50 families with diphtheria there was more than one case. It did not seem in an investigation of 500 cases of pseudodiphtheria that secondary cases were any less liable to occur where the primary case was isolated than where it was not. It is considered that where two cases occurred in a family together, or within a short period of each other, they may have been due to some common exposure rather than to direct contagion. Park believes that in the majority of cases of pseudodiphtheria the streptococci concerned are those already existing in the healthy throat, and that the presence of certain infectious diseases and exposure to cold, dampness, and insanitary surroundings, are among the exciting causes. While not denying the possibility of contagion, he considers that the degree of communicability is only moderate and of minor importance. Even admitting the possibility of contagion, he argues that the very slight mortality in uncomplicated pseudodiphtheria justifies the rule of the Health Department in not keeping this class of cases under supervision after they have been proved bacteriologically not to be true diphtheria. The question of isolation is thus left to the discretion of the attending physician.

That a comparatively small number of cases of membranous croup are due to streptococci has already been stated. Woods has reported two cases of croupous conjunctivitis following measles, in which streptococci, but no Loeffler bacilli, were found.

Persistance of Diphtheria Bacilli in the Throat During and After Convalescence from Diphtheria.—During the ten months preceding May, 1894, Park with his coworkers in the Health Department of New York has examined 752 cases of diphtheria with reference to the length of time the diphtheria bacilli remain in the throat. Cultures were made at the beginning of the disease, and again at short intervals after disappearance of the exudate, until the throat was found free of diphtheria bacilli. The custom was to make the second culture three days after the complete disappearance of the membrane, and then to repeat the culture as long as necessary every fourth or fifth day. In 325 of these 752 cases the diphtheria bacilli disappeared within three days after the complete disappearance of the exudate; in 427 cases the diphtheria bacilli persisted for a longer time, viz.: in 201 cases for 5 to 7 days; in 84 cases for 12 days; in 69 cases for 15 days; in 57 cases for 3 weeks; in 11 cases for 4 weeks, and in 5 for 5 weeks. In a case recently communicated to the writer by Park the bacilli were found 7 weeks after disappearance of the membrane.

The virulence of the diphtheria bacilli after recovery is reported by Park in 14 cases, the bacilli being obtained at intervals of 10 to 44 days after the inception of the disease Eight guinea pigs died within 40 hours, and one in each of the following periods: 60-70 hours, 5 days, 8 days, 9 days, 11 days, and 14 days. One guinea pig survived after extensive local necrosis. He concludes that the diphtheria bacilli which persist in the throat after an attack of diphtheria are always virulent for some time. In those exceptional cases in which the bacilli persist for a very long time they are found occasionally to lose their virulence a few days before their final disappearance, while in other cases they retain their virulence to the end. In one case the bacilli were virulent 8 weeks after the onset of the disease and 7 weeks after the disappearance of the membrane. That the cases themselves do not seem so liable to spread diphtheria is probably largely on account of the relatively small number of the bacilli in the convalescent throats, as compared with those showing the lesions of diphtheria. Reference has already been made to the rule of the Health Department to keep cases of diphtheria under supervision until the diphtheria bacilli have disappeared, and to practise disinfection as soon as they have vanished from the throat.

Park reports that thorough irrigation of the throat and nose with 1 to 4000 bichloride of mercury solution every few hours was attended by disappearance of the bacilli within three or four days after disappearance of the false membrane in from one-half to two-thirds of the cases. In the remaining cases the bacilli persisted from four to twenty days, notwithstanding the application of the bichloride solution. The duration of persistance of the bacilli with irrigation with simple salt water was three days less than with bichloride irrigation.

Where cultures cannot be made it is recommended to continue the isolation of diphtheria patients for at least three weeks after the disappearance of the membrane.

Morse investigated twenty-five cases of diphtheria with reference to the length of time the diphtheria bacilli persist in the throat and nose. He found the average duration of their presence after disappearance of the membrane to be ten days, and this to be the same for both throat and nose, although they were found longer in the throat in some cases, and in the nose in others. The bacilli disappeared in one case the day after the throat was clear; in three, three days after, and in one, four days after. In two cases in which the bacilli were never found in the nose, they remained in the throat ten and seventeen days respectively. In other cases they were present: in the throat thirty-seven days, in the nose thirty-six days; in the throat twenty-two days, in the nose eighteen days; in the throat ten days, in the nose seventeen days; and in both twenty-seven days. The bacilli obtained after ten days were found of usual virulence; in other cases of this group the virulence is not recorded. Patients are not permitted to leave the hospital until the diphtheria bacilli have disappeared from both throat and nose.

Presence of Diphtheria Bacilli in Healthy Throats of Persons Exposed to Diphtheria.—An interesting investigation has been carried out by Park to test the frequency with which the healthy throats of children contain diphtheria bacilli in families where a case of diphtheria exists and where little or no isolation is undertaken. The throats of fourteen families were investigated, in which there were forty-eight children. In 50 per cent of these virulent diphtheria bacilli were found; 40 per cent developed later, to a greater or less extent, the lesions of diphtheria. It is noted in Park's paper that, in these families, the conditions were the best possible for the transmission of the bacilli from one to the other. In families where the case of diphtheria was well isolated, the bacilli were found in less than 10 per cent of the children.

The cases, as well as others to be mentioned presently, in which virulent diphtheria bacilli are found, sometimes in large number, in healthy throats of persons who do not develop diphtheria, prove that virulent diphtheria bacilli may be present in the throat and multiply there without causing any visible lesions. As has recently been pointed out with much clearness by Escherich, in order to cause diphtheria they must find susceptibility to their pathogenic action, which susceptibility may be local, or general, or both.

It is, for several reasons, of practical value to make bacteriological examinations of the healthy children's throats in families where diphtheria has developed, or who have been exposed to diphtheria, especially where isolation is defective. Experience has shown, as appears from Park's paper, that antiseptic irrigations and cleansing treatment of the throat in such cases greatly reduce the liability to develop diphtheria.

The dissemination of the disease is prevented by isolating those in whom the diphtheria bacilli are found. Doubtless, not a few cases of diphtheria of obscure causation are referable to those who without the symptoms and lesions of diphtheria carry about the virulent bacilli.

Park says:

> "All members of an infected household should be regarded as under suspicion, and in those cases where isolation is not enforced the healthy as well as the sick should be prevented from mingling with others until cultures or sufficient lapse of time give the presumption that they are not carriers of contagion."

For interesting illustrative cases and further details on this important matter, Park's paper should be consulted.

Presence of Diphtheria Bacilli in Healthy Throats Where No History Could be Obtained of Exposure to Diphtheria.—Park and Beebe examined the healthy throats of three hundred and thirty persons in New York who gave no history of direct contact with diphtheria. They found nonvirulent, but otherwise characteristic, diphtheria bacilli in twenty-four, virulent characteristic diphtheria bacilli in eight, and nonvirulent pseudodiphtheria bacilli in twenty-seven. The virulence was tested by inoculating half-grown guinea pigs with $\frac{1}{2}$ to 1 per cent of their weight of forty-eight-hour bouillon cultures grown at body temperature. The nonvirulent diphtheria bacilli produced acid in bouillon, and did not differ morphologically or in cultures from virulent diphtheria bacilli. Such bacilli are not regarded by Park as pseudodiphtheria bacilli, but as genuine diphtheria bacilli devoid of virulence. The pseudodiphtheria bacilli usually clouded bouillon and did not render it acid, and presented the usual characters of the pseudodiphtheria bacilli to be described subsequently.

The nonvirulent but otherwise characteristic diphtheria bacilli were abundant in the primary cultures from seventeen throats, and present in small number in the cultures from seven. In only one guinea pig was there appreciable reaction after inoculation. Two hundred and eighty of the cases from which the cultures were made were children under twelve years of age (bacilli in twenty-two cases), while fifty were adults (bacilli in two). The nonvirulent diphtheria bacilli persisted in four of the throats for four weeks, in one for three weeks, and in three for two weeks.

Of the eight cases in which virulent diphtheria bacilli were found, five were children in an asylum where from time to time cases of true diphtheria had occurred. Of the remaining three, one was from a house where a case of supposed croup had existed three weeks before. Two of the eight children developed diphtheria some days after making the cultures. The other six never developed any symptoms of diphtheria. The guinea pigs inoculated with the cultures died in from twenty-eight to forty hours.

From the groups of cases studied under this and the preceding headings Park concludes that virulent diphtheria bacilli are present in probably about 1 per cent of the healthy throats in New York City. Most of the persons in whose throats they exist have been in direct contact with cases of diphtheria. Many of those whose throats contain the virulent bacilli never develop diphtheria.

Occurrence of Diphtheria Bacilli Outside of the Human Body.—In his first paper Park says that cultures made from the dried stains on spreads, pillow-cases, and sheets, where soiled by the expectoration of diphtheria patients, showed in every case a few colonies, at least, of the Loeffler bacillus. The sputum of patients, though apparently free from bits of membrane, usually contained the bacilli in large number.

Important results have been obtained by Wright and Emerson, who have kindly furnished the writer an abstract of their unpublished work so far as it has yet been carried They made cultures on blood serum from the clothing, shoes, hair, and finger-nails of nurses in attendance on diphtheria patients, and also from the dust and various objects within the diphtheria ward of the Boston City Hospital. This ward contains seventy beds, which are generally nearly all occupied. The hygienic condition of the ward is stated to be good. Material, such as dust, etc., was collected for the most part on a sterilized platinum wire and applied to the surface of the culture medium. About twenty cultures in all have been made, of which five yielded Loeffler bacilli in small numbers. In all of the positive cases the bacilli isolated in pure culture were tested thoroughly as to their virulence and their morphological and cultural characters, and positively identified. In the latter respect all were characteristic diphtheria bacilli. It may be especially noted that all rendered bouillon acid within forty-eight hours. In two of the five cases the bacilli were of usual virulence, in two they were weakened in virulence, although killing guinea pigs, and in one they caused only local reaction from which the animal recovered. The virulence was tested by inoculating guinea pigs subcutaneously with $\frac{1}{2}$ to 1 per cent of their weight of twenty-four- to forty-eight-hour bouillon cultures. From eight inoculated guinea pigs which died, the characteristic bacilli were recovered in each instance, even when death occurred after some weeks.

The Loeffler bacilli were found on three shoes, each belonging to a different nurse, once on the hair of a nurse, the culture being taken from the hair above the ear, and once on a brush used in sweeping the floor of the ward.

The weakening of the virulence in two cases (in one case the guinea pig died after seven and one-half days, and in another after some weeks, bacilli from each being recovered from the seat of inoculation) and the production of only slight local reaction from which the animal recovered in the case in which the bacilli were found on the hair of a nurse, suggest attenuation of virulence by unfavorable environment.

These suggestive observations of Wright and Emerson tell their own story and need no special comment.

No observations have been reported in this country of diphtheria as a spontaneous affection of domestic animals. Abbott was unable to confirm Klein's observations of the elimination of diphtheria bacilli through the milk of cows inoculated with virulent cultures of the Loeffler bacillus.

Methods of dissemination of Diphtheria.—How manifold may be the modes of conveyance of the diphtheria bacillus, and how obscure and difficult or even impossible to trace may be the origin of some cases of diphtheria, is evident from observations which have been mentioned. Park from his rich experience summarizes and illustrates with interesting examples some of the ways in which the diphtheria germ may be transported.

Here we cannot do more than enumerate some of the sources of infection which have been established by bacteriological investigations, to wit: the pseudomembranes, exudates and discharges of diphtheria patients, both those with typical diphtheria and those with mild and little characteristic forms of the disease; infected clothing, bedding, and other objects; occupying rooms where diphtheria has existed; convalescent or recovered cases in whom the diphtheria bacilli persist; persons who have been in contact with others having diphtheria bacilli on their persons or clothing, or with infected objects, such persons sometimes carrying diphtheria bacilli in their throats for days or weeks without lesion. The investigations of Wright and Emerson show on what varied objects the diphtheria bacilli may be carried. Park traced one group of cases of diphtheria to a candy store kept by a family in which occurred a case of diphtheria. Children who bought candy at this store acquired diphtheria, and other children who came in contact at school with the healthy children of this family also developed diphtheria.

The investigations of the Health Department indicate that at present the whole tenement house district of New York is an infected area. In plotting out on a map the distribution of cases of diphtheria in New York it was interesting to note two types of epidemics, one essentially of neighborhood infection, the other in which the cases could be attributed to infection at school. It was observed that a whole school district would suddenly become the seat of scattered cases. At times, in a certain area of the city from which several schools drew their scholars, all the cases of diphtheria would occur in families whose children attended one school, the children from the other schools being for a time exempt.

In about one-half of the cases of diphtheria in New York careful inquiry failed to show connection with other cases.

Influences which increase susceptibility to diphtheria doubtless play a rôle in determining the development of the disease. The majority of adults and many children are insusceptible. What these predisposing influences are we do not know, save the influence of age. That special local conditions, the presence of throat affections and the association of the diphtheria bacillus with other microorganisms, particularly streptococci, are predisposing factors seems highly probable. It is also probable that the degree of virulence of the diphtheria bacillus and the number of bacilli received into the body must be taken into account.

We possess no evidence that the diphtheria bacillus finds a natural home outside of the human body, although it may survive for months on objects outside of the body. Park found living diphtheria bacilli on bits of membrane dried for seventeen weeks, and in blood serum cultures seven months old.

Pseudodiphtheria Bacillus.—The various questions relating to the pseudodiphtheria bacillus have been investigated by Abbott, Koplik, and Park.

In fifty-three cases of various throat affections, mostly without suspicion of diphtheria, examined bacteriologically by Abbott, he found in three bacilli indistinguishable morphologically or in cultures from the Loeffler bacillus, but entirely devoid of virulence, as tested repeatedly on guinea pigs. One of the cases was suspicious of diphtheria, there being a grayish membrane on the tonsils; one was a case of syphilitic pharyngitis without membrane, and the other was follicular tonsillitis. From a fourth case of this series with a grayish white deposit on the tonsils, Abbott cultivated a bacillus resembling the Loeffler bacillus, but somewhat thicker, growing more luxuriantly on agar, clouding bouillon and growing visibly on potato, exceptionally invisibly. This bacillus was wholly nonpathogenic for guinea pigs. As already mentioned, Abbott and his pupil, Ravenel, have found in two cases of membranous rhinitis bacilli indistinguishable from the Loeffler bacillus except that they failed to kill guinea pigs, although producing local reaction.

Abbott's conclusion is that the diphtheria bacillus varies in its virulence and that nonvirulent varieties of the bacillus occur. He says that the name pseudodiphtheria bacillus should not be applied to those bacilli which possess all of the morphological and cultural characters of the Loeffler bacillus and are devoid of virulence. These are simply nonvirulent diphtheria bacilli. He reserves the term pseudodiphthritic bacillus " for that organism or group of organisms (for there are probably several) that is enough like the diphtheria bacillus to attract attention, but is distinguishable from it by certain morphological and cultural peculiarities aside from the question of virulence."

Koplik does not seem to have met with a bacillus presenting all the morphological and cultural properties of the virulent diphtheria bacillus but devoid of virulence. He emphasizes the importance, in testing virulence, of observing the precautions especially signalized by Behring and by Escherich, as already mentioned, and gives examples showing that mistakes on this point can readily be made where old cultures and insufficient doses are used. Koplik has, however, in six cases out of a large number examined found the pseudodiphtheria bacillus essentially as described by Hoffmann, and he would limit the name pseudodiphtheria bacillus to the bacillus presenting these definite characters. This pseudodiphtheria bacillus he describes in his first article as shorter and slightly plumper than the real bacillus, but subsequently he describes it as an exact counterpart of the Loeffler bacillus in shape, size and staining peculiarities, with the exception of deeper staining with methylene blue. Actual measurements do not show any constant differences in thickness or length from the Loeffler bacillus. It grows upon blood serum, potato, and gelatin, like the genuine bacillus. Upon agar the growth is always more luxuriant, thicker and whiter than that of the real bacillus. In alkaline peptone bouillon the pseudobacillus causes in twenty-four hours diffuse clouding with abundant deposit. The reaction remains distinctly alkaline. Koplik communicates to the writer an unpublished observation to the effect that if the pseudobacillus be cultivated in closed tubes of Pasteur pattern, from which the oxygen has been absorbed by pyrogallate of potassium, there is an abundant sediment with diffuse clouding of the bouillon, and in forty-eight hours the reaction is found to be acid. Neither aerobic nor the acid anaerobic cultures are lethal to guinea pigs, even in enormous doses. Nor does the preparation of an animal for weeks by the injection of such repeated doses protect it from the effects of small doses of a fresh culture of the virulent bacillus. This was proved not only upon guinea pigs, but also upon sheep and dogs. Koplik's first article is illustrated with beautiful photographs of the real and the false diphtheria bacilli in microscopical specimens and in cultures It contains also a very satisfactory photograph of involution forms of the diphtheria bacillus.

The pseudobacillus was found by Koplik in four cases, already mentioned, with inflammation of the tonsils, without the real bacillus. In two cases of lacunar tonsillar diphtheria virulent typical diphtheria bacilli persisted in the crypts, in one case for three weeks, and in the other case for two weeks. At the end of these periods the virulent bacillus gave place to a nonvirulent bacillus indistinguishable morphologically from the real bacillus, but presenting the cultural characters above described of the pseudobacillus, this bacillus being found abundantly in exactly the same situations in which the virulent bacillus previously existed. These nonvirulent bacilli persisted in the tonsillar lacunae up to the sixth week after the onset of the illness in one cases. Koplik suggests the possibility that under the influence of the lymph and leucocytes of the tonsils, the virulent bacillus was transformed into the nonvirulent form. He considers, however, the relationship between these two bacilli to be still an open question, but upon the whole he seems to be of the opinion that they are distinct species. When found, the pseudodiphtheria bacilli were abundant in the cultures. Koplik's results may be said to be confirmatory of those of Escherich.

Park found, in 20 cases diagnosticated as diphtheria, the bacilli to be virulent in all, but in three of these cases the cultures from the first colony selected, although characteristic, were not virulent, while from other colonies in the same cases they were fully so. As already stated, Park used in testing virulence forty-eight hours' broth cultures injected in doses of one-quarter to one-half or even one per cent of the body weight of the guinea pig—selecting, where possible, young guinea pigs. Park, therefore, confirms the observation of Roux and Yersin as to the possible coexistence of virulent and nonvirulent bacilli in the same throat. He considers that in suspicious cases one should not be content with cultures from a single colony, if this should prove not to be virulent.

In this connection it may be mentioned that Park found no definite relation between the virulence of the cultures and the severity of the case. Nor was he able in an extended research to discover a relationship between the size of the bacillus and its virulence such as has been claimed by some investigators. He states that the results of 1613 cultures in which this factor was carefully noted in relation to the severity of the case indicate that in New York the great majority of cases of diphtheria yield in cultures bacilli of medium size which are characteristic in shape and in manner of staining, and that in a moderate number the bacilli are much longer, and in about an equal number they are much shorter. Both clinical histories and animal experiments showed that as long as in shape and staining the bacilli are characteristic, nothing as regards their virulence can be judged from their size. Those bacilli, on the other hand, which are short and stain uniformly with methylene blue usually turn out to be of the pseudodiphtheria type which have no virulence in animals.

It has already been mentioned that Park found exceptionally in cases in which diphtheria bacilli persisted in that throat a long time after recovery from diphtheria, attenuation of virulence to a point in which the cultures, although producing local reaction, failed to kill guinea pigs.

We have cited already the investigations of Park and Beebe upon the healthy throats of 330 persons who gave no history of direct contact with diphtheria. In 24 of these they found bacilli indistinguishable from the diphtheria bacillus except by absence of virulence, which was tested by injecting forty-eight hour broth cultures in doses of one-half to one per cent of their weight into half-grown guinea pigs. In 19 of these 24 cases the bacilli were grown in broth. In all of these the bacilli formed acid by their growth in forty-eight hours, sometimes more, sometimes less acid than control cultures of virulent bacilli. In 13 the broth culture was entirely characteristic, like that of the virulent bacillus, while in 6 the culture became more or less clouded, an effect which is exceptionally produced also by the virulent bacillus. No difference was found as to the growth on blood serum. Upon agar the growth was typical of that of the virulent bacillus in 11 cases, while from 7 the bacilli grew in a less typical manner, but always in ways seen occasionally with the virulent form. Of the guinea pigs inoculated with cultures from 15 of the cases, in only one was there any appreciable local reaction, and this subsided within four days. A very slight degree of immunity seemed to be afforded to some of the guinea pigs by the injection.

In 27 cases the pseudodiphtheria bacillus, which, as well as the genuine diphtheria bacillus, has been most satisfactorily photographed in coverslip specimens and cultures by Park, was found presenting in general the characters described above. Park describes this bacillus as shorter and thicker and more uniform in size and staining than the virulent Loeffler bacillus. It clouded and formed alkali in bouillon in the first forty-eight hours, grew more luxuriantly on agar, and was not pathogenic. Park would confine the name pseudodiphtheria bacillus to the bacillus or group of bacilli presenting these general characters.

Park, as well as Abbott, therefore, recognizes a nonvirulent form of the diphtheria bacillus, and contends that bacilli indistinguishable from the diphtheria bacillus except by absence of virulence, should not be called pseudodiphtheritic. He admits certain morphological and cultural variations of the true diphtheria bacillus, such as variations in size, occasional clouding of bouillon, exceptional luxuriance of growth on serum and agar, etc., but the production of acid in bouillon he regards as essential to the diagnosis of the diphtheria bacillus.

The pseudodiphtheria bacillus, as defined above, is regarded by Park as a species distinct from the genuine diphtheria bacillus, either in its virulent or its nonvirulent state. Park calls attention to the confusion introduced into the discussions on the pseudodiphtheria bacillus by the fact that some have given this name to bacilli indistinguishable from the diphtheria bacillus except by absence of virulence, whereas others have worked with the genuine pseudodiphtheria bacillus, which presents cultural and morphological differences from the genuine diphtheria bacillus.

It would seem to be established, therefore, by the investigations of Abbott and Park, that a bacillus in no way distinguishable from the diphtheria bacillus except by absence of virulence exists. Although other investigators have included this nonvirulent but otherwise characteristic diphtheria bacillus under the designation pseudodiphtheria bacillus, they contend that this name is inappropriate for this bacillus, which should be considered as the genuine diphtheria bacillus, which may present all degrees of virulence down to complete harmlessness, as tested on guinea pigs. This nonvirulent bacillus has been found only in a comparatively small number of cases, these cases being chiefly without characteristic lesions of diphtheria. Occasionally the virulent and nonvirulent diphtheria bacilli are found side by side in diphtheria, and occasionally the virulent bacillus has given place to the nonvirulent bacillus during or after recovery from diphtheria.

Abbott, Koplik, and Park would limit the name pseudodiphtheria bacillus to bacilli which, although bearing a resemblance to the diphtheria bacillus, differ from it not only by absence of virulence toward guinea pigs but also by cultural characteristics.

Abbott considers that there is a group of bacteria which in this sense may be called pseudodiphtheritic, and that they are distinct species from the diphtheria bacillus. Koplik describes with much detail a special bacillus observed by him in six cases, which he considers to be identical with the original pseudodiphtheria bacillus of Hoffmann, and to which he would limit the name pseudodiphtheria bacillus. This bacillus grows on agar more luxuriantly than the genuine diphtheria bacillus, and it does not change the reaction of alkaline glucose bouillon in aerobic cultures, although it produces acid in anaerobic cultures. While not expressing a positive decision, he seems inclined to regard this bacillus as distinct in species from the genuine diphtheria bacillus.

Park has probably worked with the same bacillus, at least the same as the one described by Koplik in his first article, and he also would limit the name pseudodiphtheria bacillus to this bacillus, or to a group of bacilli presenting these several characteristics, if, as is probable, such a group exists. He considers that this bacillus, or these bacilli, differ from the genuine diphtheria bacillus at least as much as the colon bacillus differs from the typhoid bacillus. The pseudodiphtheria bacillus was found in only a small number of cases out of the many examined, and appeared to have nothing to do with diphtheria.

Summary.—This paper is a report of the results of the bacteriological study of diphtheria in the United States up to May, 1894, so far as these results are of interest for the purposes of this Congress. The investigations were made by various men in New York, Baltimore, Boston, and Philadelphia.

Some of the more important conclusions may be summarized as follows:

1. The Health Department of New York has undertaken the bacteriological examination of all cases of suspected diphtheria in that city, unless objection is made by the attending physician or unless it is not deemed advisable to disturb the patient by such examination. The methods employed are described in detail. During the year ending May 4, 1894, cultures were made from 5611 cases of suspected diphtheria. The results have proven satisfactory, and are utilized not only for diagnosis, but also to control the supervision and isolation of the cases.

2. Of 6156 cases of suspected diphtheria in New York and Boston, 58½ per cent were proven bacteriologically to be true diphtheria—or, if we include only those cases in which the bacteriological examination was considered to be entirely satisfactory—of 5340 cases, 67½ per cent were true diphtheria. These were pseudomembranous inflammations of the throat and air passages uncomplicated for the most part with scarlet fever.

3. At least 80 per cent of the cases of membranous croup in New York were diphtheria, and only 14 per cent were shown not to be diphtheria.

4. Fifteen cases of fibrinous rhinitis and 4 cases of primary and exclusively nasal diphtheria were all due to the diphtheria bacillus.

5. Various forms of atypical diphtheria, many without membrane, and with the characters of simple catarrhal angina and follicular tonsillitis, are described.

6. Instances of unusual localizations of the diphtheria bacillus, as in the middle ear, in wounds, ulcers, abscesses, conjunctivae, lungs, heartvalves, and the distribution of the bacilli at autopsies of human beings and of guinea pigs dead of diphtheria, are described.

7. The various bacteria found associated with the diphtheria bacillus, the most important pathogenic forms being streptococci, staphylococci, and *Diplococcus lanceolatus,* are considered.

8. In general the great majority of cases of pseudomembranous anginas in scarlet fever are due to streptococci, but where diphtheria is prevalent and opportunities are favorable for exposure to diphtheria a large proportion may be due to the diphtheria bacillus. The statistics in Baltimore and in Boston present interesting contrasts in illustration of this point. Four cases of diphtheria complicating typhoid fever are described.

9. The name pseudodiphtheria is applied to pseudomembranous inflammations of the throat and air passages not caused by the diphtheria bacillus. The most important and common microorganism in pseudodiphtheria is *Streptococcus pyogenes,* but other bacteria may be the cause. The mortality in these affections is low in private practice, being 1.7 per cent in 408 consecutive cases in New York. In hospitals it may be as high as 25 per cent. Death is generally due to some complication, the most important complications being scarlet fever, membranous laryngitis, and bronchopneumonia. The disease seems to be only slightly, if at all, contagious. For this reason, and on account of the low mortality in uncomplicated cases, those cases which are proved bacteriologically not to be true diphtheria are not kept under supervision by the Health Department in New York. Until such proof suspicious cases are treated as diphtheria.

10. Of 752 cases of diphtheria in New York, the diphtheria bacilli in 325 disappeared within three days after the complete disappearance of the exudate. In 427 cases the bacilli persisted for a longer time, viz., in 201, for from five to seven days; in 84, for twelve days; in 69, for fifteen days; in 57, for three weeks; in 11, for four weeks; and in 5, for five weeks. In one case, virulent bacilli were found seven weeks after disappearance of the exudate. The cases are kept under supervision until the bacilli have disappeared. Sometimes they disappear first from the nose; at other times, first from the throat.

11. In fourteen families, with forty-eight children, where little or no isolation of a case of diphtheria in each family was undertaken, virulent diphtheria bacilli were found in 50 per cent of the children, of whom 40 per cent later developed diphtheria. The bacilli were found in less than 10 per cent of the children in families where the case of diphtheria was well isolated.

Antiseptic irrigation and cleansing treatment of the throat lessens the liability of those thus exposed to develop diphtheria.

All members of an infected household should be regarded as under suspicion, and where isolation is not enforced the healthy as well ar the sick should be prevented from mingling with others until cultures or sufficient lapse of time give the presumption that they are not carriers of contagion.

12. Diphtheria bacilli may be present and multiply in the throat without causing symptoms or lesions. They must find susceptibility to their pathogenic action in order to cause diphtheria.

13. In three hundred and thirty persons who gave no history of direct contact with diphtheria, virulent diphtheria bacilli were found in eight, of whom only two subsequently developed diphtheria. Bacilli indistinguishable morphologically or in cultures from the diphtheria bacillus, in-

cluding the formation of acid in forty-eight hours in bouillon, but entirely devoid of virulence, were found in twenty-four of these persons, in most of these instances in large numbers. The pseudodiphtheria bacillus was found in twenty-seven.

14. Instances are given in which the diphtheria bacilli were found on various objects outside of the human body, viz., bedclothing soiled with discharges of diphtheria patients; the shoes and the hair of nurses in attendance on diphtheria patients, and a brush used in sweeping the floor of a diphtheria ward.

15. Some of the various ways in which the diphtheria germ is transported are summarized.

16. A bacillus in no way distinguishable in morphology or in cultures, including the formation of acid in bouillon, from the usual diphtheria bacillus, but devoid of virulence, exists. The virulence was tested by injecting into half-grown guinea pigs ½ to 1 per cent of their weight of forty-eight-hour bouillon cultures. This bacillus, although it has been called by some investigators the pseudodiphtheria bacillus, should not be so designated. It is the genuine diphtheria bacillus devoid of virulence. It was met with in a comparatively small number of cases out of a large number examined. Exceptionally, it may occur together with the virulent diphtheria bacillus in diphtheria, and occasionally it takes the place of the virulent bacillus during or after recovery from diphtheria. In several instances it was found in healthy throats.

The name pseudodiphtheria bacillus should be confined to bacilli which, although resembling the diphtheria bacillus, differ from it not only by absence of virulence, but also by cultural pecularities, the most important of the latter being greater luxuriance of growth on agar and the preservation of the alkaline reaction of bouillon cultures. The pseudodiphtheria bacillus may render bouillon cultures acid in forty-eight hours when grown anaerobically. The pseudodiphtheria bacillus in this sense was found in a number of cases, but not frequently. It is probably of different species from the genuine diphtheria bacillus, and is without diagnostic importance.

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THE TREATMENT OF DIPHTHERIA BY ANTITOXIN[1]

I shall endeavor in this paper, after a brief historical introduction, to present some of the more important general considerations bearing upon the treatment of diphtheria by antitoxic serum, together with statistics of results already reported, with the expectation that those who are to follow in this discussion before the Association will be able to offer the results of personal experience in the application of the new remedy.

In July, 1889, Babès and Lepp, in an article entitled "Recherches sur la Vaccination Antirabique,"[2] published results of experiments undertaken to solve the question, "Whether the fluids and cells of animals which have been rendered by vaccination immune have not becomes vaccines and capable of protecting also other organisms?" The results of these experiments showed that the blood of dogs thoroughly vaccinated against rabies, when injected into susceptible animals, conferred a certain amount of protection against the effects of subsequent inoculation with the rabid virus, and appeared capable of preventing the development of rabies even when the injection of the immune blood was made immediately after the reception of the virus. The authors concluded that "one must admit the possibility of vaccinating with the fluids and cells of animals which have been rendered refractory to the disease."

The first publication clearly demonstrating the principles of serum-therapy was made by Behring and Kitasato on December 4, 1890, in an article in the "Deutsche medicinische Wochenschrift," entitled, "Ueber das Zustandekommen der Diphtherie—Immunität und der Tetanus—Immunität bei Thieren." Although in this article the immunizing and curative property of the blood and blood serum of artificially immunized animals was demonstrated only for tetanus, the application of the same principle to diphtheria was indicated in the same article and in a second paper by Behring in the following number of the same journal.

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[1] This paper is based upon the address made by the writer at the opening of the discussion on this subject before the Association of American Physicians, Washington, May 30, 1895. It is considerably expanded beyond the limits of the address, and I have endeavored to bring it up to the date of sending it to the printer (July, 1895).

Tr. Ass. Am. Physicians, Phila., 1895, X, 312-384.

[2] Babès and Lepp: Annales de l'Institut Pasteur, July, 1889.

Richet and Hericourt are sometimes quoted as the first experimenters to show that the blood of animals is capable of conferring protection upon susceptible animals, but their work has no reference to modern serum-therapy, as their experiments were made with the blood of dogs which had not previously been vaccinated or treated in any way.

The first public announcement of the demonstration of the power of the blood serum of animals artificially immunized against diphtheria to protect and cure susceptible animals inoculated with the diphtheria bacillus or its poison was made by Behring in the report of experiments made by himself and Wernicke, and communicated to the Seventh International Congress of Hygiene and Demography, held in London in August, 1891. There followed in 1892 the article by Behring and Wernicke,[3] in which these experiments were fully described, and which sets forth the fundamental principles underlying serum-therapy of diphtheria.

The first trial of immune serum in the treatment of human diphtheria was made in von Bergmann's clinic, in Berlin, in the autumn of 1891. This trial, together with those made in 1892 by Henoch, in Berlin, by Heubner, in Leipzig, and in the Institute for Infectious Diseases in Berlin, were of a tentative nature and made with weak serum and insufficient doses.

In was not until early in 1893 that Behring succeeded in obtaining antidiphtheric serum equalling the strength of even his so-called normal serum, of which sixty times the strength is that of the weakest Behring serum at present in use. In April, 1893, Behring referred to 30 cases treated with this normal serum. Of these cases, 11 treated in the Institute for Infectious Diseases were reported in detail by Kossel.

From this period on Behring and Ehrlich succeeded in obtaining healing serum of greater and greater strength, until in August, 1893, Ehrlich and Wassermann obtained from goats healing serum twenty to sixty times the strength of Behring's normal serum. At the Eleventh International Medical Congress, held in Rome (March 29 to April 5, 1894), Heubner reported the results of his experience with the serum-treatment of human diphtheria. His observations, however, were made on cases treated with much weaker antitoxin than is now recognized as suitable.

In April, 1894, Ehrlich, Kossel, and Wassermann reported briefly the results of treatment of 220 cases in six Berlin hospitals, the inception of the treatment in these cases dating from June, 1893, but the great majority of the cases occurring after December, 1893. These cases, with additional ones making a total of 233 cases, with a fatality of 23 per cent, were reported more fully in an article by Kossel in the "Zeitschrift für Hygiene," in July, 1894. The era of serum treatment of human diphtheria by approximately sufficient doses of antitoxin really begins with this publication of Ehrlich, Kossel, and Wassermann in April, 1894, although, even in this series of cases, according to later statements of Ehrlich and Kossel, a large number of the cases were treated with quantities of antitoxin which we now consider to be insufficient.

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[3] Behring and Wernicke: Ueber Immunisirung und Heilung von Versuchthieren bei der Diphtherie. Zeitschrift für Hygiene, Bd. xii.

In an address before the Berlin Medical Society, on June 27, 1894, Katz reported the results of antitoxin treatment dating from March 14, 1894, with Aronson's serum from horses, on 128 cases of diphtheria in Baginsky's service. In the discussion on this address four weeks later, Baginsky completed the series of cases up to 163, with a mortality of 12.9 per cent, and Aronson stated that similarly favorable results had been obtained by Ganghofner in Prague and Escherich in Graz.

In 1893 and the first half of 1894 various articles appeared concerning the preparation of antitoxin, the best methods of estimating its strength, the proper immunizing and therapeutic doses, and similar questions. Since August 1, 1894, Behring's serum prepared at Höchst has been for sale.

It is evident from this brief historical summary that the general principles of serum-therapy of diphtheria were fully established, and its application to human beings in active operation before Roux delivered his memorable address on the subject at the Eighth International Congress of Hygiene and Demography, held in Budapest, September 1-9, 1894, three years after Behring's original communication to the preceding congress in London. Roux, however, presented the subject with such clearness and force, and with such an array of convincing and carefully analyzed statistical evidence, that the attention of the great body of physicians throughout the world, who had paid little heed to the previous work, was arrested, and the question of the healing power of diphtheria antitoxin became and has continued to be the foremost medical question of the day. From September, 1894, onward the supply of antitoxin from various sources (not all of equally trustworthy character) has become more and more accessible to physicians, and each succeeding months has given birth to a large number of articles on the serum-therapy of diphtheria from various parts of the world.

Unless one denies absolutely the causal relation of the Loeffler bacillus to diphtheria, it must be admitted that the treatment of this disease by antitoxin rests upon a sound experimental basis. The only notable opponent of the view that the Loeffler bacillus is the cause of diphtheria is Hanseman. His arguments, which have been well answered by C. Fraenkel, are equally applicable to the acceptance of the etiological relations of the cholera spirillum, the tubercle bacillus, and many other specific bacteria of infectious diseases. It is not probable that anyone here sides with Hanseman in this matter, so that it is unnecessary to rehearse the arguments, which, in my judgment, are conclusive that the Loeffler bacillus is the cause of diphtheria.

The laboratory does not furnish any more impressive experiments than those which demonstrate the power of antitoxic serum to prevent and to cure the disease caused in animals by inoculation with the diphtheria bacillus or its poison. The serum arrests the spread of the local process and abates the symptoms of general toxaemia. These experiments prove beyond question that this healing serum possess properties which are directly and powerfully antagonistic to the toxic action of the diphtheria bacillus, and there is no good reason to doubt that under similar circumstances this antagonistic power, so readily and surely and uniformly demonstrable in the case of lower animals, will manifest itself also in human beings. The only question, and that, of course, an important one in this connection, is: To what extent the conditions in the treatment of experimental diphtheria by antitoxin are or can be made similar to those in the therapeutic application of the same agent to human diphtheria?

Although it is true that the lower animals are not susceptible, or only very exceptionally susceptible, to natural infection with the Loeffler bacillus, still there is in my opinion identity in essential points—anatomical, clinical, and etiological—between experimental diphtheria and uncomplicated human diphtheria. The assertion sometimes made that spreading pseudomembranous inflammations resembling those of diphtheria cannot be produced experimentally in animals by inoculation with the Loeffler bacillus is an error, as I have repeatedly had opportunity to demonstrate by intratracheal inoculations of kittens and rabbits. It is rarely in our power to reproduce experimentally in one species of animal the exact counterpart of a disease caused in another by natural modes of infection, but in the case of diphtheria the resemblance is closer than in most of our attempts to reproduce such diseases by inoculation of their specific germs.

But even if the anatomical and clinical characters of experimental diphtheria are believed by some to differ more widely than I think they do from those of human diphtheria, there remains as the most important point, regarding the matter here under discussion, the conclusive demonstration that in uncomplicated human diphtheria, no less than in experimental diphtheria, the local inflammation at the site of infection is caused by the growth of the Loeffler bacillus, and the lesions of internal parts and the systematic symptoms are due to the absorption of a toxic substance or of substances formed by this bacillus. It would be difficult to understand why an agent with the specific property of neutralizing in the bodies of animals the effects of these toxic substances should be unable to neutralize in human beings similar effects of the same toxic substances, provided this agent can be administered in the proper dose and at the right time.

Dosage and timely administration are factors of prime importance in determining the efficacy of antitoxic treatment. It is our inability to conform to the demands of these factors which has rendered thus far the treatment of tetanus in human beings by antitoxin disappointing. The tetanus antitoxin can be produced by methods similar to those employed in making the diphtheria antitoxin and of a power expressed in immunizing units greater than that of the diphtheria antitoxin. No less striking than in diphtheria are the laboratory experiments in the prevention and cure of artificial tetanus by administration of the tetanus antitoxin, but in this case the dose of antitoxin required to check the disease increases so enormously with increase in the size of the animal, on the one hand, and with the lapse of time after reception of the virus, on the other hand, that we meet herein most serious obstacles to the successful application of this agent in the treatment of human tetanus.

It has been shown experimentally by Behring, Boer, Roux, and others, that as regards both of these points the conditions are far more favorable for the treatment of diphtheria by its antitoxin than in the case of tetanus. In an animal at a certain time after reception of the tetanus poison the theoretically efficacious dose of the tetanus antitoxin may be a millionfold greater than that required for simple immunization, a quantity too large to administer, whereas the effective dose of the diphtheria antitoxin at relatively the same period may be increased only eight or tenfold. Doubtless the great advantage which we have in the treatment of human diphtheria by antitoxin as contrasted with tetanus is that we are able to recognize and treat the former disease before the production and absorption of a serious quantity of poison.

Only clinical experience can determine what practical difficulties there may be in the way of the successful employment of antitoxic serum in the treatment of human diphtheria; but there is no doubt in my mind that the results derived from experiments on animals justify, nay, demand, the most careful and thorough trial of the new method of treatment upon human beings.

We have no certain knowledge as to the nature of the substances called antitoxins nor as to their mode of action. This is not, however, an argument against their therapeutic employment, for we have no positive knowledge as to the mode of action of many of our therapeutic agents. There are two prominent theories as to the mode of action of the diphtheria antitoxin. The one may be called the chemical, and the other the vital theory. The chemical theory is that the antitoxin directly neutralizes in a chemical sense the toxins. This seemed to be the natural interpretation of the fact that the injection into susceptible animals of a mixture in suitable proportions of the antitoxin and the toxin is harmless, but Buchner and Roux have shown that this earlier view is incorrect, and that by selecting animals of greater susceptibility, or by increasing the natural susceptibility of an animal, the presence of active toxin in the mixture can still be demonstrated. The experimental evidence, therefore, is in favor of the other theory, viz., that the antitoxin acts through the agency of the living body, and probably in the sense that it renders the cells tolerant of the toxin. The results of the treatment of human diphtheria with antitoxin also speak in favor of this vital theory.

If, as seems probable, the curative effects of the healing serum are brought about through the agency of the living cells of the body, we can understand why these effects will not follow the introduction of the serum with the certainty and precision of a chemical reaction. The cells must be in a condition to respond in the proper way to the introduction of the antitoxic serum. For one reason or another this responsive power may be in abeyance. It may be weakened by intense or prolonged action of the diphtheria poison, or by other previous or coexistent disease or by inherent weakness, or there may even be some individual idiosyncrasy which hinders the customary response of the cells to the antitoxin. Clinical experience shows that cases of diphtheria inherently refractory to timely treatment with antitoxic serum are most exceptional, if indeed they occur at all.

There is some evidence in favor of the view that while antitoxin may exert its protective action upon certain groups of cells, other cells, as for example the nerve cells, either by their nature or on account of such influences as I have mentioned, may not be equally protected against the toxin. There is also the possibility that the antitoxin may neutralize the effects of certain toxins and not of others present in diphtheria.

Antitoxic serum exerts no bactericidal effect upon the diphtheria bacillus, although when administered in proper quantities sufficiently early in the disease it arrests the spread of the local inflammation which is caused by the bacillus. Virulent bacilli may persist in the throat days and even weeks after recovery following injection of antitoxin.

One of the most important characters of antitoxin is that it requires a definite quantity of this substance to neutralize the effects of a definite quantity of toxin. In animals the curative dose of antitoxin stands in a definite quantitative relations to the size and susceptibility of the individual and to the amount and intensity of the poison in the system. We have no precise method of determining how much and how virulent the poison may be in a given case of human diphtheria, nor how susceptible to the toxin the patient may be. The dosage of antitoxin, therefore, in human diphtheria is empirical, the main factors determining it being the age of the patient, the assumed duration of the disease up to the time of administering the remedy, and the apparent severity of the disease. As the healing serum is expensive and is capable of inducing unpleasant symptoms, it is desired not to give an excessive quantity. Under these circumstances it may readily happen that an insufficient dose is given and that the administration must be repeated. The general rules regarding the dosage of antitoxin are sufficiently well known not to require mention here, and I speak of this matter only to indicate that because a patient may have received a dose, or even two or more doses of antitoxin, this furnishes no absolute guarantee that a quantity of antitoxin adequate to neutralize the effects of the toxin has been given. We now know that in the early period following introduction of the treatment entirely insufficient doses were given.

Both experiments on animals and clinical experience demonstrate that the earlier antitoxic serum is administered after the inception of the disease the better are the chances of recovery. It is usually impossible to rescue the lives of guinea pigs by means of antitoxin if the treatment is delayed longer than forty-eight hours after inoculation with an amount of diphtheria poison fatal to these animals in four or five days, although the duration of life may be considerably prolonged. In human beings the conditions are different, but, as will appear from the statistics to be presented, the evidence is conclusive that the superiority of serum treatment over all other methods is most strikingly manifested in the results of the cases in which the antitoxin is given not later than the third day of the disease. Although in many cases the treatment is beneficial when the antitoxin is administered in larger doses at a later period of the disease, the importance of beginning the treatment at the earliest possible date, without waiting to determine by cultures whether or not the Loeffler bacillus is present, cannot be too strongly emphasized.

It is, of course, often impossible to meet this demand for early treatment, as cases of diphtheria are frequently not seen or recognized by the physician, particularly in hospital practice, until after several days' duration of the disease and when grave symptoms have already developed. It is, moreover, in many cases difficult or impossible to determine how long the disease has existed when it is first seen by the physician.

The fact that the benefits of antitoxin treatment become more and more doubtful the further the disease has progressed and the graver the lesions and symptoms, renders more difficult the collection and analysis of absolutely convincing statistics in favor of the treatment. The accusation is sure to be brought that many of the cases which have responded promptly to early treatment, and these for reasons which have been stated will form a large contingent of the successful cases, were mild cases which would have recovered equally by other methods of treatment. This objection can be fully met only by large series of statistics collected from many epidemics, at different times and in various localities.

The bacteriological study of human diphtheria has disclosed several points important to bear in mind in determining the value of antitoxic treatment. The Loeffler bacillus has been found in healthy throats, although only very exceptionally, unless the person has been exposed to diphtheria. This same bacillus may cause all grades of inflammation of the throat from a mild erythematous angina to the gravest pseudomembranous inflammations. There has resulted a conflict, not yet settled, between the clinical and the bacteriological diagnosis of diphtheria. As regards these diversities of effect, however, the conditions pertaining to the diphtheria bacillus are in no way different from those relating to many other pathogenic bacteria, as, for example, the pneumococcus, the streptococcus, the cholera spirillum, and even the tubercle bacillus, all of which may be found on healthy mucous membranes and may exert their pathogenic activity with all degrees of intensity. Inconvenient as these facts may be, they must be recognized, and they require a readjustment of previously adopted boundary lines of diagnosis. It would, of course, be absurd to say that a person who harbors in his healthy throat Loeffler bacilli has diphtheria, just as it would be equally ridiculous to consider a person infected with the pneumococcus or the streptococcus when these latter bacteria are present under similar conditions. But it is no less absurd to limit the application of the term "diphtheria" only to those higher degrees of pathogenic action of the Loeffler bacillus characterized by spreading pseudomembranous inflammations and general toxaemia.

But while the boundaries of the domain of diphtheria have thus been widened by the inclusion of cases not presenting the ordinary clinical characteristics of diphtheria, in another direction they have been restricted by the exclusion of some cases which on clinical grounds would be diagnosed as diphtheria, but which by bacteriological examination are proved to be caused by other bacteria than the Loeffler bacillus.

The statement is sometimes made that 25 to 30 per cent, or even a larger percentage, of the clinical diphtherias are not genuine diphtherias in the bacteriological sense, but this statement is quite misleading. These figures are based upon the bacteriological examination of large numbers of cases in which there was simply more or less suspicion of diphtheria. They do not relate generally to a large number of cases presenting unmistakable anatomical and clinical characteristics of diphtheria. They are derived from the routine examinations for boards of health and children's hospitals of suspected cases of diphtheria. When one considers that in some cases of diphtheria repeated, painstaking examination, microscopical and cultural, by a skilled bacteriologist is required for the detection of the diphtheria bacillus, it is evident that less reliance is to be placed upon these statistics *en gros* than upon many smaller series reported by bacteriological experts. Of the statistics of the latter character there are many which show that in the series of cases examined (including in each series from a dozen to over three hundred cases), from 90 to 100 per cent of the clinical diphtherias are due to the Loeffler bacillus. Our experience in Baltimore has been that not over 5 per cent of the cases which the clinician would confidently diagnose as diphtheria are false diphtheria or diphtheroid. These latter figures relate, of course, to primary diphtheria, and not to the pseudomembranous anginas complicating scarlet fever and other infectious diseases, a large proportion of which are not referable to the Loeffler bacillus.

I shall consider subsequently in this article the influence which the control of the clinical diagnosis of diphtheria by bacteriological examination is likely to have upon fatality statistics of this disease.

There is an important difference between experimental diphtheria and many cases of human diphtheria, a difference of great significance in determining the scope of efficiency of treatment by antitoxic serum. Our experimental diphtheria is a pure, uncomplicated infection in which only the diphtheria bacillus and its toxins are concerned. On the other hand, in many cases of human diphtheria there are complications and mixed infections due to other microorganisms, against which, when fully developed, the diphtheria antitoxin is powerless. The most common and dangerous complicating microorganism is *Streptococcus pyogenes*. Bacteriological examinations of fatal cases of diphtheria demonstrate in a large proportion of cases the invasion and pathogenic effects of this most common of all secondary invaders. The confidence with which some observers, particularly of the French school, classify their cases of diphtheria into pure and mixed infections, on the sole basis of the bacteriological examination of the exudate in the throat, does not seem to me justifiable. The complete microscopical and cultural examination of this exudate will, in practically all cases, reveal the presence of other bacteria, and usually of streptococci, besides the Loeffler bacillus. But as these other bacteria are common or regular inhabitants of the healthy throat, their mere presence in this situation is not conclusive evidence that they are engaged in pathogenic action. The abundance of these other bacteria may afford some indication as to their rôle, but of greater importance is their demonstration in situations where they are not normally present.

Reiche,[1] in 42 autopsies on cases of diphtheria in which the Loeffler bacillus had been demonstrated during life, made cultures from the kidney and spleen. In 64.3 per cent of these cases streptococci and staphylococci were found in the kidney or the spleen, and in 45.2 per cent streptococci were found alone. These cocci must have reached these organs through the circulating blood. He found streptococci in the kidney in one case, which died on the second day of the disease, and positive results were obtained also on the third and fourth day. These results are evidently of much significance in indicating the frequency and the earliness of invasion of complicating microorganisms in diphtheria and the resulting obstacles to uniformly favorable results of antitoxin treatment.

But the chief evidence in favor of mixed infection must be sought during life in the character of the lesions and symptoms, although these may be misleading. There is also evidence that the failure of a case of diphtheria to respond in the usual way to the timely injection of a sufficient dose of antitoxic serum is an indication of complications and mixed infection.

The opinion is entertained by Roux, Martin, and other French writers that bronchopneumonia, one of the most common and serious complications of diphtheria, is due to a large extent to local unhygienic conditions which can be guarded against. Thus they attribute the frequent occurrence of bronchopneumonia in some groups of their cases to the infection of the hospital wards with the bacteria causing pneumonia, and claim that by improved sanitary conditions this complication may be to a large extent eliminated. Further investigations are needed to determine to what extent this view as to the causation of bronchopneumonia is justified, but it can scarcely be doubted that this complication is often the result of invasion of the lower air passages and the lungs by bacteria which are regularly present in the throat, and whose activity is likely to be manifested in this way in many cases of diphtheria, independently of the local sanitary conditions.

Without doubt the remedial rôle of diphtheria antitoxin is materially restricted by its inability to combat developed streptococcus sepsis, bronchopneumonia, and other complications referable to secondary infection, or to stop impending suffocation by immediate removal of mechanical obstacles in the form of false membranes in the air passages; but the antitoxic serum is the most powerful agent which we possess to prevent the development of these complications and secondary infections. The timely administration of the healing serum, by antagonizing the effects of the Loeffler bacillus, antagonizes in large part the causes of the increased susceptibility to secondary infection, and thus greatly lessens the frequency of their occurrence.

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[1] Reiche: Centralblatt f. innere Medicin, 1895, No. 3.

In considering the obstacles in the way of cure of diphtheria by antitoxin the self-evident fact should not be forgotten that this remedy cannot restore cell life which has already been seriously damaged by the action of the diphtheria bacillus or its poison. The researches of Oertel upon human diphtheria, and those of Flexner and myself upon experimental diphtheria, demonstrate that the toxins of the diphtheria bacillus are most powerful poisoners of cells, the internal lesions of pure diphtheria being especially characterized by widely distributed areas of cell death. We have no way of gauging accurately at any given period of the disease the extent of the damage already inflicted upon the cells of the body. If the nerve-cells or their axis-cylinders have already been so damaged that paralysis must follow, or the cardiac nerve-cells or muscular fibres have been similarly injured, or the renal epithelium so affected that degeneration and nephritis ensue, the administration of antitoxin cannot restore those cells which are already on the way to degeneration and death.

This irretrievable damage to cell life may be present for a considerable time before we are able to recognize its effects. P. Meyer detected pathological changes in the peripheral nerves as early as the third day after the onset of diphtheria and before paralysis was manifest. The occurrence of paralysis, including cardiac paralysis, after antitoxin has been administered even thus early in the disease, cannot, therefore, necessarily be attributed to failure of this agent to neutralize toxin developed after its injection.

Having now considered the experimental basis and the theories of action of antitoxic treatment, the importance of early administration and sufficient dosage, and certain etiological and pathological characters of human diphtheria to be borne in mind in estimating the scope of the treatment, let us turn to the examination of the evidence which has hitherto been published concerning the efficacy of the antitoxic treatment of human diphtheria. This evidence is of two kinds: first, the general impressions of clinicians who have had opportunity to observe the effects of antitoxin administered in a number of cases of diphtheria, and, second, the mortality statistics of cases treated with antitoxin.

Unquestionably great value attaches to the impressions and conclusions of careful clinical observers as to the merits of therapeutic agents. Baginsky has said that naked figures are so little the expression of the endless variations of clinical observation, of all those forunate and unfortunate accidental circumstances which pertain to the constitution and nutrition of the patient, and of the complications and difficulties which may bring danger in a mild attack or lead to a successful issue an apparently severe attack, that to the clinical observer such figures appear of little value in comparison with the treasure-house of his accumulated experience. And it is to his experience of many years in the same hospital and on similar clinical material that Baginsky repeatedly recurs in his monograph, "Die Serum-therapie der Diphtherie," in support of his favorable conclusions as to the healing power of antitoxin, and this in spite of the fact that his statistical results, leading to the same conclusions, are based upon a larger number of cases than those of any other single observer yet published, and are among the most convincing of the statistical reports. In explaining why, at the end of ten months' trial of antitoxin, he has determined to commit himself to a definite judgment in its favor, he says:

> "The reasons for this are to be found in the continual repetition of improvement and recovery of severe cases which previous experience indicates would have terminated fatally, and, furthermore, in the outcome of an involuntary experiment with interruption of the use of the serum for a period on account of failure in its supply. During this period the mortality of our patients immediately rose again to its former height. The improvement in the general condition of the patients imparts to our diphtheria wards an entirely different character from the former one. That this is not due to any change in the character of the clinical material, to milder forms of the disease, was unfortunately demonstrated by the observations in the months of August and September, when, as by a single blow, we were transported back to the old times, to the same melancholy picture of children deeply prostrated and often in vain struggle with death."

In August and September the supply of antitoxin failed.

The published testimony of those who have had the largest opportunity to study the therapeutic effects of antitoxin is overwhelmingly in its favor. In no less favorable terms than those of Baginsky are expressed the opinions of such observers of high reputation and extended experience as Heubner, von Widerhofer, von Ranke, Ganghofner, Escherich, Bokai, and the Physicians of the Hôpital des Enfants Malades and Hôpital Trousseau, in Paris. These observers have reported already in detail over 2300 cases of diphtheria treated with antitoxin.

Many of those who have reported smaller series of cases, and a few who have reported as many as a hundred cases, have expressed themselves with much caution or have not ventured any final judgment, although in most of these reports the results appeared to be favorable to the new treatment. An example of this conservative position is that of Vierordt, who says that a final decision as to the value of antitoxic serum is not to be expected in the immediate future, as such decision requires a long series of observations in different epidemics and on varied clinical material.

Antitoxic serum is a new and strange remedy, but the effects which follow its injection in individual cases are not new and strange. Nothing happens which the physician may not have occasionally seen to happen in cases treated in the ordinary way. In severe as well as in mild cases of diphtheria he may have seen an apparently progressive local process quickly arrested and the general symptoms promptly abated. But why should anything new and strange happen after the administration of antitoxin? Cure by antitoxin is cure by Nature's own remedial agent. That which is new and strange is the frequency with which in case after case the timely injection of antitoxin promptly arrests the local inflammation and checks the constitutional disturbance.

Recovery following treatment by antitoxin is such a natural kind of recovery that in any given case the physician may readily have the feeling that the same thing might have happened without the use of the remedy. We can, therefore, understand why it should be those with the largest experience in the treatment of diphtheria by antitoxin who are most decided in expressing their opinion as to its beneficial effects. The very fact that the mode of cure is such a natural one, and unattended by peculiar phenomena, is an obstacle to drawing positive conclusions from a small number of observations, even if these appear most favorable.

That there should be wide diversity in the percentage of cures in reports of different observers is, of course, to be expected when we consider the varied character of the cases treated and the importance of early administration of antitoxin. It may happen that a series of cases is made up so largely of advanced and complicated diphtherias at the time when the antitoxin treatment is begun, that the beneficial effects of the treatment are not apparent. It is, on the whole, remarkable that there should have been so few reports in which the fatality has not been materially diminished during the period of administration of antitoxin.

There are only a few writers who on the basis of personal experience (in no instance a large one) have expressed an opinion unfavorable to antitoxin. Kohts may be mentioned as one, who on the basis of 47 cases treated with serum, with 29.1 per cent deaths among the tracheotomized, and 7.6 per cent among the nontracheotomized, finds such apparently favorable results no better than by other methods of treatment.

So far, then, as the testimony of physicians based upon their clinical experience is concerned, this, as I have already said, is overwhelmingly in favor of the antitoxic treatment, wherever this experience in its employment has been a large one. Those with less experience are often even more enthusiastic; but many of these, in view of their limited experience, are wisely conservative and a few are hostile to the new treatment.

But general clinical impressions, convincing as they may be to the individual receiving them, may not be equally convincing to others. They do not furnish any strict scientific proof of the value of a therapeutic agent. If antitoxin really exerts any specific curative action in diphtheria, this must be apparent in the figures of fatality statistics of this disease; and it is only by such statistics, much as they may be decried by some, and difficult as it may be to guard them from errors of interpretation, that a strictly scientific demonstration of the efficacy of antitoxin in the treatment of diphtheria can be brought.

The possible fallacies of interpretation belonging to fatality statistics in general apply in no small measure to those of diphtheria. The case-mortality from diphtheria varies within wide limits according to the more or less severe character of the prevailing epidemic, according to the season of the year, according to the age, according to the method of treatment, in cities and in country districts, etc. Statistics of case-mortality from hospital practice will differ widely from those from private practice, and each of these will differ from the general case-mortality returns from cities. Nor does each of these three classes of statistics represent a uniform material. The material of one hospital may consist very largely of cases of diphtheria admitted in an advanced stage of the disease, or of laryngeal cases sent for operation, while that of another hospital may contain a much larger proportion of cases admitted in early stages of the disease. In general the fatality of diphtheria in hospital practice is higher than that of private practice, as would be expected from the later stage of the disease in which the patients generally enter the hospital; but to this rule there are many exceptions. In some hospitals the patients are all children, in others there may be a considerable proportion of adults with diphtheria. In private practice among the poor, patients may be first seen by the physician frequently in as advanced stages of the disease as in hospitals, and the conditions for successful treatment, and particularly for intubation or tracheotomy, are less favorable for this class of private patients than for hospital patients.

Still other reasons might be given for the lack of uniformity of diphtheria statistics from different sources; but enough has been said to show that as regards the question which interests us here each report of a series of cases treated with antitoxin requires its own special consideration and analysis, and is not comparable with reports from other sources relating to a different class of cases.

The larger the number of cases embraced in the statistical tables the greater becomes the mutual compensation of such differences as those mentioned, and, therefore, the more trustworthy are the conclusions derived from the statistics; but in collecting the statistics of the general fatality of diphtheria treated with antitoxin it has seemed to me important, for the reasons which have been mentioned, that the tables should contain for each report, as far as possible, statements of the total number of cases treated with antitoxin, of the number and percentage of deaths, of the previous or simultaneous fatality, and of the class of cases, whether in hospital or in private practice. I have also analyzed the cases so far as practicable according to the ages of the patients and according to the day of the disease on which antitoxin treatment was begun. It has seemed to me of especial interest to consider the fatality in operated and unoperated cases. There are, of course, many other points of view which it would have been interesting to consider in the statistical study of the cases reported; but it has seemed to me that the analysis already indicated should suffice to determine the main question at issue, namely, the specific curative power of antitoxin, as well as certain other questions.

It is scarcely ten months since antitoxin has been used by more than a very few favored physicians, and it is a much shorter time since its use has become at all general. In this comparatively short time there have, however, been published more or less definite reports of the results of the treatment in at least 15,000 cases. I have collected 82 reports from 80 different sources, containing 7166 cases. These are presented in Table I. This collection of cases is by no means complete, as I have consulted only the more readily accessible journals; but it is believed to include all of the more important reports. I have not included any reports of single cases, as these are often to illustrate some special point, nor any reports of series of cases less than ten. Indeed, only four reports with less than twelve cases in the group have been included in the tables. Nor have I made use of such merely general published statements without detail, as that there have been treated in France up to the end of December 2700 cases, with a mortality of 16 per cent; in Austria, outside of Vienna, 950 cases, with a mortality of 15.7 per cent; in Croatin and Slavonia, 428 cases, with a mortality of 10.8 per cent in Berlin hosptials, 1500 cases, with a reduction in fatality of one-half, etc. Eulenburg has recently (July 15, 1895) made a provisional report concerning the collective investigation inaugurated by the "Deutsche medicinische Wochenschrift," by sending out cards to be filled out by physicians regarding their results in the treatment of diphtheria with and without serum. Up to the date of the report the cards returned embraced 10, 240 cases of diphtheria, of which 5790 were treated with serum and 4450 without serum The total mortality of the former group was 9.5 per cent; that of the latter group 14.7 per cent. No further details of this investigation have as yet been published, and these cases are not included in my tables. (See foot-note, page 311.)

I have entirely avoided the duplication of cases, so far as I can determine.[5]

[5] This duplication of cases appears in several of the published statistics, especially of the Berlin statistics. Thus the cases reported by Schubert, Voswinckel, Canon, and Weibgen appear in the report of Ehrlich, Kossel, and

There has been no selection whatever of cases. All of the reports of the characters described which came to my notice are included, although many of the early Berlin cases (contained in statistics of Körte, Sonnenburg, and Hahn in my tables) and some of the others were treated with entirely insufficient doses of antitoxin, and some observers have purposely selected, especially at the time when there was little serum to be had, only severe cases for treatment. Kohts' 47 cases could not be inserted, as he does not give the number of deaths in the report which I have seen.

The reports are of unequal value. Some present full and precise details of each case, or of the group of cases, with statements as to previous or simultaneous fatality in the same class of cases in the same locality, whereas others are meagre and unsatisfactory.

Some reports are based upon the bacteriological control of the clinical diagnosis; others upon the clinical diagnosis uncontrolled by bacteriological examination. In general, the statistics from the larger hospitals relate to cases in which the Loeffler bacillus was demonstrated, whereas many of the reports from private practice are without bacteriological examination. A few, notably Leichtenstern and Wendelstadt, purposely base their observations upon cases in which the diagnosis is purely clinical without bacteriological control.

I shall take this opportunity to consider the influence which the requirement of the bacteriologist, that the clinical diagnosis of diphtheria should be controlled by a bacteriological examination in testing the efficacy of antitoxin treatment, is calculated to have upon the character of statistics intended to show the value of the treatment. It is a favorite criticism of these statistics that the bacteriological, as distinguished from the purely clinical, diagnosis of diphtheria will operate in favor of a low fatality in antitoxin statistics, and that therefore it is unfair to compare these statistics with those which are based upon the uncontrolled clinical diagnosis of diphtheria. Some of the critics would have us believe that the antitoxin statistics, on the one hand, contain a large proportion of cases of mild inflammation of the throat with Loeffler bacilli, but which no clinician would recognize as diphtheria; and, on the other hand, exclude a large proportion of fatal pseudomembranous inflammations of the throat and air passages which clinically would be regarded as diphtheria.

Wassermann, and partly in the reports of Körte, Sonnenburg, and Hahn, sometimes twice repeated. Most of these cases are included without duplication in my table, although they were treated with insufficient doses to a large extent. The cases attributed to Virchow, Aronson, and Katz in some statistics are included in those of Baginsky.

In most of the statistical reports from hospitals on antitoxin treatment the statement is expressly made, and it is apparent from the description of the cases, that they do not represent anything else than the usual run of cases of diphtheria as they have regularly for years past presented themselves at the same hospitals. The mild diphtheric sore throats without clinical evidences of ordinary diphtheria are not likely in any large number to be recognized at all as diphtheria, and still less likely to find their way into general hospitals, from which most of the statistics are derived. Where, as in the statistics of Baginsky and others, sufficient detail concerning each case is reported to enable the reader to form an intelligent estimate of the character and severity of the case, it is evident that affections without the customary anatomical and clinical characters of diphtheria do not enter into the statistics.

It is erroneous to say that the antitoxin statistics are not based upon the clinical diagnosis of diphtheria. The diagnosis is clinical, but with subsequent bacteriological control. The cases are admitted to the hospital with the clinical diagnosis of diphtheria, and the healing serum is or should be at once administered without waiting for the result of the cultures from the throat. Soltmann has been justly criticised for delaying the injection of antitoxin until after the bacteriological examination was completed.

The assumption that nonmembranous anginas and tonsillitis containing Loeffler bacilli figure to any appreciable extent in these statistics is without warrant of facts.

There are treated, of course, together with severe cases, many mild cases with small patches of membrane on the tonsils or in the throat; but such cases are clinically diphtheria or certainly ought to be suspected of diphtheria by the clinician. It is important that such cases, when caused by the Loeffler bacillus, especially in young children, should be treated by antitoxin, for not a few such cases when untreated develop into severe cases, sometimes suddenly into laryngeal diphtheria. Kurth, for example, relates a case in which a twin brother of a child ill with diphtheria was found to present small membranous patches on the tonsils, which during two weeks of observation would at times disappear, and which did not apparently make the child ill. Loeffler bacilli were demonstrated, but the parents would not consent to the injection of serum. At the end of fourteen days laryngeal diphtheria suddenly developed. The injection of antitoxin was followed by recovery in four days. This is simply a type of not a few cases which are regarded as suddenly developed laryngeal diphtheria.

If, as is doubtless true, in some hospitals a larger number of cases are now received for serum treatment in earlier stages of diphtheria than formerly, this is not because the bacteriological diagnosis has supplanted the clinical, but because the importance of early inception of serum treatment has been justly emphasized. The recognition of mild and very mild cases of diphtheria is not a discovery of the bacteriologist, but has long been known to physicians, nor is it a peculiarity of the fatality statistics of cases treated by antitoxin that such mild cases are included in the statistics. They appear equally in previous fatality statistic of diphtheria. Mosler, for example, reports 313 cases of diphtheria with a fatality of 14.5 per cent, treated during a year in the Greifswald clinic before the introduction of serum, and there are numerous other statistics showing that mild cases often preponderate in previous fatality statistics of diphtheria. Nor is the comparison in all of the reports made with former statistics of cases in which the diagnosis is without bacteriological control. In several reports the comparison is with the results of cases in which the Loeffler bacillus was demonstrated, but were not treated with serum.

As regards the exclusion from antitoxin statistics of cases presenting the clinical characters of diphtheria without the Loeffler bacillus, it is evident, from what has previously been said, that, with thorough bacteriological tests, this can affect only a very small number of cases of unmistakable primary clinical diphtheria. Of the cases concerning which the clinician is in doubt, a considerable proportion are not diphtheria by bacteriological examination, which alone can decide the question. Although some of the nondiphtheric, pseudomembranous cases are very grave affections, their general mortality is much lower than that of genuine diphtheria. The exclusion from the fatality statistics of diphtheria of the pseudomembranous cases without Loeffler bacilli is, therefore, the exclusion of a generally milder class of cases, as has been repeatedly demonstrated, and the result is to assign a higher and not a lower fatality to the remaining cases. A few examples taken from reports in Table I will suffice to demonstrate this. From the statistics of Roux, Martin, and Chaillou 118 pseudomembranous cases treated with serum were thrown out because they were proven subsequently to be devoid of Loeffler bacilli. The fatality of these cases was only 8.5 per cent, whereas, in the 300 remaining cases which contained Loeffler bacilli and were injected with serum, the fatality was 26 per cent. In Sevestre's and Meslay's statistics 29 cases without Loeffler bacilli, but treated with serum, gave a fatality of 3.4 per cent, as opposed to a fatality of 10 per cent in the treated cases containing Loeffler bacilli. A similar difference appears in other reports. The serum has no curative influence on pseudomembranous inflammations not caused by the Loeffler bacillus.

In the best reported statistics information is afforded as to these various points, and the reader can learn the ration of apparently mild cases and the number and results of the diphtheroid cases.

Although only those statistics which are based upon the thorough bacteriological examination of the cases treated can lay claim to entire accuracy, the benefits of antitoxic treatment are clearly apparent in reports based upon the uncontrolled clinical diagnosis of diphtheria. Of course, in ordinary general practice it is not to be expected that the diagnosis will rest upon a bacteriological examination; but it should be understood that in the absence of such examination there must be occasional instances of apparent failure of antitoxin which would be found explicable had a bacteriological examination been made.

In many reports the percentage of deaths in the cases treated with antitoxin is corrected by excluding cases evidently hopeless on admission, or dying within twenty-four hours after commencement of the treatment. These corrected percentages are usually very materially lower than the rates based on all of the deaths. For example, if the cases dying within twenty-four hours after injection of antitoxin be excluded, the percentage of deaths in Roux, Martin, and Chaillou's cases becomes 21.5 instead of 26; in Baginsky's 13.5 instead of 15.6; in von Widerhofer's 14.3 instead of 23.7; in Vierordt's 14.6 instead of 25; in Lebreton's and Magdelaine's 10.8 instead of 12; in Moizard's and Perregaux's 11.3 instead of 14.7; in Sevestre's and Meslay's 6.6 instead of 10; in Bokai's 18.3 instead of 25.5, etc. I have, however, not used these reduced percentages, although in many instances it might with propriety have been done. That statistics in my tables, therefore, do not give, in many instances, as favorable percentages for antitoxin as may be justly claimed; but on the other hand, they are more properly comparable with the previous or simultaneous fatality rates from diphtheria, these being based upon the total number of deaths in all of the cases treated. I have aimed to avoid the accusation of selection of cases or unfair manipulation of the figures.

In the majority of the reports the cases treated are all, or nearly all, of the cases of diphtheria which were admitted to the hospital or came under observation during the period of treatment; but in some it is expressly stated that, in consequence of the cost and the scarcity of the healing serum, mild cases and evidently hopeless cases did not receive the serum. There is no evidence of selection of mild cases in order to obtain results favorable to antitoxin.

The percentages in the column headed "Previous Fatality" are those given by the writers for diphtheria not treated with antitoxin. In some instances they relate to the average fatality for a series of preceding years, in some to the minimum and the maximum fatality for several years, in some to the simultaneous fatality or the fatality during a period of interruption in the supply of serum. In all instances they are the case mortality-rate

TABLE I

Fatality of Cases of Diphtheria Treated with Antitoxin

Number of cases of diphtheria treated with antitoxin, the number and percentage of deaths, and the previous fatality in 82 reports

Reporter	Cases	Deaths	Previous fatality	References
1. Roux, Martin, and Chaillou.	300	78 (26 p. ct.)	50 p. ct.	Ann. de l'Institut Pasteur, Sept. 1894 (in Hôpital des Enfants Malades).
2. Kossel	117	13 (11.1 p. ct.)	52–61 p. ct.	Deutsche med. Wochen., 1894, p. 946 (hospital).
3. Körte	121	40 (33.1 p. ct.)	45.1 p. ct.	Berliner klin. Wochen., 1894, p. 1039 (hospital).
4. Sonnenburg	95	16 (16.8 p. ct.)	27.6 p. ct. (period of interruption of serum treatment.)	Deutsche med. Wochen., 1894, p. 930 (hospital).
5. Hahn	205	49 (24 p. ct.)	41 p. ct.	Ibid., 1895, Vereins-Beilage, p. 2 (hosp.).
6. Baginsky	525	83 (15.6 p. ct.)	41 p. ct.	Die Serumtherapie der Diphtherie, von Dr. Adolf Baginsky. Berlin, 1895 (hospital).
7. Heubner	207	27 (13 p. ct.)	48–52 p. ct.	Reported at 13ten Congress für innere Medicin, München, April 2, 1895. Münchener med. Wochen., April 9, 1895 (hospital).
8. von Widerhofer	300	71 (23.7 p. ct.)	50 p. ct.	Ibid. (hospital).
9. von Ranke	96	19 (19.7 p. ct.)	42.2–56 p. ct.	Ibid. (hospital).
10. Stintzing	59	12 (20.1 p. ct.)	25 p. ct.	Ibid. (hospital).
11. Rauchfuss	100	34 (34 p. ct.)	55 p. ct.	Ibid. (hospital).
12. von Mering	74	4 (5 p. ct.)	30 p. ct.	Ibid. (hospital).
13. von Noorden	81	19 (23 p. ct.)	45 p. ct.	Ibid. (hospital).
14. Schröder	63	8 (12.7 p. ct.)	30–37 p. ct.	Ibid. (hospital).
15. Vierordt	63	16 (25 p. ct.)	41–67 p. ct.	Deutsche med. Wochen., 1895, p. 169 (hospital).
16. Rumpf	22	2 (8 p. ct.)	13–28 p. ct.	Münchener med. Wochen., Nov. 20, 1894 (hospital).
17. Lebreton and Magdelaine.	258	31 (12 p, ct.)	50 p. ct.	Le Bulletin Médical, 1895, No. 10 (Hôpital des Enfants Malades).
18. Le Gendre	17	3 (17.6 p. ct,)	50–60 p. ct.	Bull. et Mémoires de la Soc. Méd. des Hôpitaux de Paris, Dec. 20, 1894 (Hôpital Trousseau).
19. Moizard and Perregaux.	231	34 (14.7 p. ct.)	50–60 p. ct.	Journ. de Méd. et de Chirurg., Dec. 25, 1894 (Hôpital Trousseau).
20. Sevestre and Meslay.	150	15 (10 p. ct.)	50–60 p. ct.	Le Bulletin Médical, 1895, No. 18 (Hôpital (Trousseau).
21. Ganghofner	110	14 (12.7 p. ct.)	43.6–78.2 p.c.	Prager med. Wochen., 1895, Nos. 1, 2, and 3 (hospital).
22. Soltman	89	13 (14.6 p. ct.)	27.2 p. ct.	Deutsche med. Wochen., 1895, No. 4 (hospital).
23. Bokai	176	42 (24 p. ct.)	53.5–67.5 p.c.	Ibid., 1895, No. 15, and Wiener med. Presse, 1895, No. 12 (hospital).
24. Escherich	51	5 (9.5 p. ct.)	....	Abstract in Münchener med. Wochenschrift, 1895, No. 7 (hospital),
25. Gnändinger	27	11 (40.7 p. ct.)	36–45.5 p. ct.	Wiener klin. Wochenschrift, 1895, No. 1 (hospital).
26. Monti	25	1 (4 p. ct.)	....	Wiener med. Wochen., 1895, Nos. 4 and 5 (hospital).
27. Heim	27	6 (22 p. ct.)	52.5 p. ct.	Ibid., 1895, No. 4 (hospital).
28. Unterholzner	31	8 (25.8 p. ct.)	66.7 p. ct.	Ibid. (hospital).
29. Bäumler	26	2 (7.7 p. ct.)	....	Münchener med. Wochen., 1894, p. 1062 (hospital).
30. Börger	30	2 (6.66 p. ct.)	20 p. ct.	Deutsche med. Wochen., 1894, No. 48 (hospital).
31. Hilbert	11	0	....	Ibid., 1894, Vereins-Beilage, p. 142 (hospital).
32. Hager	25	1 (4 p. ct.)	....	Centralbl. f. innere Medicin, 1894, No. 48 (private practice).

TABLE I—*Continued*

Reporter	Cases	Deaths	Previous fatality	References
33. Moeller	76	27 (35.5 p. ct.)	....	Ibid. (hospital).
34. Kuntzen	25	3 (12 p. ct.)	....	Deutsche med. Wochen., 1894, No. 49 (hospital).
35. Schmidt	14	3 (21.4 p. ct.)	....	Ibid., 1894, No. 52 (private practice).
36. Seiz	27	1 (3.6 p. ct.)	30.6 p. ct.	Therapeut. Monatshefte, Dec. 1894 (private practice).
37. Charon	13	4 (30.8 p. ct.)	....	Annales de la Soc. Royale des Sci. Médicales et Naturelles de Bruxelles, 1894, t. iii, p. 337 (hospital).
38. Washbourn, Goodall, and Card.	72	14 (19.4 p. ct.)	36–41.8 p. ct.	British Med. Journ., Dec. 22, 1894 (hospital).
39. Seitz	35	2 (5.7 p. ct.)	....	Abst. in Münchener men. Wochen., 1895, No. 12 (hospital ?).
40. Pavlik	13	1 (7.7 p. ct.)	....	Wiener med. Presse, 1895, Nos. 1 and 5 (private practice).
41. Handler	32	5 (15.6 p. ct.)	....	Ibid., No. 6 (private practice).
42. Herringham	18	3 (16.7 p. ct.)	....	British Med. Journ., Dec. 22, 1894 (hospital).
43. Caiger	30	8 (26.6 p. ct.)	30 p. ct.	Ibid., Dec. 29, 1894 (hospital).
44. Hall	11	3 (27.3 p. ct.)	31.25 p. ct.	Ibid., Jan. 19, 1895 (hospital).
45. Tirard and Willcocks.	10	1 (10 p. ct.)	38 p. ct.	The Lancet, Jan. 19, 1895 (hospital).
46. Ruffer	274	37 (13.5 p. ct.)	....	Cases treated in four London hospitals, Brit., Med. Journ., Feb. 2, 1895.
Epidemic in Trieste:				
47. Private practice.	72	5 (6.5 p. ct.)	43.8-62.6 p.c.	Das Oesterreichische Sanitätswesen, Jan. 3, 1895.
48. Hospital	180	40 (22.2 p. ct.)		
49. Blumenfeld	50	2 (4 p. ct.)	....	Wiener klin. Wochen., 1895, No. 3 (private practice).
50. Witthaucr	36	5 (14 p. ct.)	....	Therapeut. Monatshefte, Feb. 1895.
51. Dreyfus	78	15 (19.3 p. ct.)	50 p. ct.	Lyon Médical, Feb. 3, 1895 (hospital).
52. Simon	16	2 (12.5 p. ct.)	....	La Médecine Moderne, Feb. 6, 1895 (private practice).
53. Malvoz	15	1 (7 p. ct.)	....	Le Scalpel, Feb. 17, 1895.
54. Gougenheim	125	12 (9.5 p. ct.)	21.1 p. ct.	Ann. des mal. de l'Oreille, du Larynx et du Pharynx, 1895, No. 5; abst. in the Med. News, June 15, 1895 (hospital).
55. Rapmund	100	7 (7 p. ct.)	20–30 p. ct.	Zeitschrift f. Medizinal-Beamte, Feb. 15, 1895 (collective investigation of use of antitoxin in private practice in district of Minden.
56. Schüller	32	1 (3.1 p. ct.)	....	Allgem. med. Central-Zeitung, 1895, No. 88 (private practice).
57. Grünfeld	12	1 (8.3 p. ct.)	....	Abst. Schmidt's Jahrb., 1895, Bd. 246, p. 37 (private practice).
58. Schaewen	15	0	....	Berliner klin. Wochenschrift, 1895, No. 10 (private practice).
59. Heidenhain	24	3 (12.5 p. ct.)	....	Ibid. (private practice).
60. Risel	114	9 (7.9 p. ct.)	....	Deutsche med. Wochenschrift, 1895, No. 10 (hospital and private practice).
61. Weiland	20	0	....	Ibid. (from private practice of six physicians in Baden).
62. v. Muralt	58	2 (3.4 p. ct.)	....	Correspondenzbl. f. Schweizer-Aerzte, 1895, No. 5 (hospital).
63. Blattner	38	9 (23.6 p. ct.)	....	Abst. in Münchener med. Wochen., March 5, 1895 (hospital).
64. Gerloczy	55	15 (27.3 p. ct.)	....	Abst. ibid. (hospital).
65. D'Espine	60	6 (10 p. ct.)	....	Rev. méd. de la Suisse Romande, (April 20, 1895 (40 cases in hospital).
66. Mya	17	2 (11.8 p. ct.)	....	Wiener med. Blätter, 1895, p. 760 (hospital).

TABLE I—*Continued*

Reporter	Cases	Deaths	Previous fatality	References
67. von Engel........	39	10 (25.5 p. ct.)	50 p. ct......	Prager med. Wochenschrift 1895.
68. Fischer..........	225	35 (15.5 p. ct.)	....	New York Med. Record, April 6, 1895 (hospital, consultation and private practice).
Biggs:				
69. Treated in city by sanitary inspectors.	255	40 (15.69 p.ct.)	25–35 p. ct...	Ibid., April 20, 1895.
70. Treated at Willard Parker Hospital.	164	45 (27.4 p. ct.)	32 p. ct......	
71. Lennox Browne..	45	2 (4.4 p. ct.).	34 p. ct......	Le Bull. Méd., 1895, No. 21 (hospital).
72. Codd and Whitehouse,	11	4 (36.4 p. ct.)	....	Brit. Med. Journ., May 18, 1895 (hospital).
73. Winkfield........	22	4 (18.2 p. ct.)	36 p. ct......	Ibid., May 11, 1895 (hospital).
74. Horowitz........	21	1 (4.8 p. ct.)	10.7 p. ct....	Abst., in Münchener med. Wochen., May 7, 1895 (hospital).
75. Sigel............	100	12 (12 p. ct.).	40–50 p. ct...	Abst., ibid., May 21, 1895 (hospital).
76. Howard..........	40	3 (7.5 p. ct.)	....	The Medical News, June 1, 1895 (private practice).
77. Van Nes..........	52	12 (23 p. ct.).	36–48 p. ct...	Deutsche med. Wochen., June 6, 1895 (hospital).
78 Leichtenstern and Wendelstadt...	123	25 (20.3 p. ct.)	30.9 p. ct....	Münchener med. Wochen., June 11, 1895 (hospital).
79. Kurth............	97	10 (10.3 p. ct.)	....	Deutsche med. Wochen., July 11, 1895 (35 hospital cases).
80. Timmer..........	31	6 (19.4 p. ct.)	....	Abst. in Deutsche Medizinal-Zeitung, June 10, 1895 (hospital).
81. Cases treated in Cartagena.	156	21 (13.5 p. ct.)	....	Abst. in Brit. Med. Journ., July 6, 1895.
82. Mason............	306	81 (26.4 p. ct.)	45–52 p. ct...	Reported to Assoc. Amer. Phys., May 31 1895. Abst. in Med. News, June 15, 1895 (hospital)
Total, 82 reports..	7166	1239 (17.3 p. ct.)		

of diphtheria in the hospital or locality from which the cases treated with antitoxin are derived. The arrangement of the reports is only in part chronological. Following the references to the articles it is stated, so far as could be ascertained, whether the cases were in hospital or in private practice.

The fatality of 7166 cases of diphtheria treated with antitoxin was 17.3 per cent.

The previous or simultaneous fatality of cases not treated with antitoxin is stated in 45 reports. These reports contain 5406 cases treated with antitoxin, with 1008 deaths, a fatality of 18.6 per cent. Estimating the number of deaths in these cases upon the basis of the previous or simultaneous fatality for each group (taking the lowest figures given), there would have been 2279 deaths, or 42.1 per cent. There was, therefore, an apparent reduction of case-mortality by the use of antitoxin of 55.8 per cent.[6]

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[6] The report of Fürth concerning the results of serum treatment in the medical and surgical clinics at Freiburg was published too late to be included in my tables; 100 cases were treated, with a fatality of 12 per cent. During the five preceding years the fatality from ordinary treatment fluctuated between 31 and

It appears from Table I that of 7166 patients with diphtheria treated with antitoxin 1239, or 17.3 per cent died. Among these cases are included many treated during the early period after the first introduction of the treatment with entirely insufficient doses. There are also included a large number of cases dying from complicating diseases not referable to diphtheria, or dying within twenty-four hours after beginning the treatment, cases which cannot properly be regarded as indicating failure of the serum treatment. The great bulk of the statistics come from children's hospitals. Under these circumstances, indeed, from any point of view, the fatality derived from Table I of cases treated with antitoxin is very low. There is, however, no standard of comparison for the fatality in this entire group of cases. It cannot be compared with fatality statistics from hospitals nor with those from private practice. The table contains at least five to six times as many cases from hospital practice as from private practice. The ratio of deaths to all cases, therefore, is greater than could be expected from the returns of all cases similarly treated in cities; but even in comparison with municipal fatality statistics of diphtheria during the prevalence of mild types of the disease the percentage of deaths is very low. This strikingly low fatality in itself speaks strongly in favor of the efficiency of the serum treatment.

In 46 reports contained in the table the previous or simultaneous percentage of deaths from diphtheria not treated with antitoxin is given for the same hospital or locality in which were the cases treated with antitoxin. These reports contain 5406 cases of diphtheria treated with antitoxin with 1008 deaths, or 18.6 per cent. If we calculate the number of deaths in each series of these cases upon the basis of the previous fatality, selecting the lowest percentage given, we have 2279 deaths, or 42.1 per cent. There is, therefore, on this estimate, by the use of antitoxin, an apparent reduction in the number of deaths of 55.8 per cent. There must be a much greater difference between the characters of the cases composing the two groups compared than appears from the statements of the writers and the details of the cases described, if this striking reduction in fatality is not due in large part to the serum treatment.

If we separate the hospital cases from those in private practice, we obtain from 61 reports of Table I 5777 cases of diphtheria treated with antitoxin in hospitals. These furnished 1081 deaths, giving a percentage of 18.7.

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49 per cent, averaging 39 per cent. The same average existed during the seven months of the year corresponding to the period during which the antitoxin was used. There was laryngeal involvement in 43 cases, and tracheotomy was performed in 31, with 11 deaths (35.4 per cent). In previous years tracheotomy had been required in 46.2 per cent of the cases, with a mortality of 70.4 per cent. (Abstract in The Medical News, August 17, 1895.)

Although this is not an unheard-of fatality of diphtheria in hospitals, it is most exceptional, and I am not aware that an equally low fatality has been observed in hospitals receiving large numbers of cases of diphtheria in children. The fourth column in Table I gives the percentages observed in many such hospitals.

There are 41 reports which give for the same hospital the previous percentage of deaths from diphtheria not treated with antitoxin. These furnish 4899 cases treated with antitoxin, with 944 deaths, or 19.3 per cent. If we calculate the number of deaths which would have occurred among these cases had the percentage of previous fatality obtained, selecting the lowest percentages given, there would have been 2130 deaths, or 43.5 per cent. The apparent diminution in the number of deaths as the result of serum treatment is, according to this estimate, 55.6 per cent. If we had selected only the larger and most carefully analyzed and satisfactory statistics from the principal hospitals—in large part children's hospitals—there would have been in over 3000 cases an apparent reduction in fatality of 60 per cent.

There may occur considerable differences in the annual fatality from diphtheria in a hospital during a series of years; but such differences between the minimum and the maximum fatality as that just noted between the actual and the estimated fatality are most exceptional. In the Friedrichshain hospital in Berlin there has been observed a difference of 28 per cent in the annual fatality from diphtheria. The largest difference observed in the surgical clinic in Berlin during ten years was that between 43.2 per cent in 1888 and 58.5 in 1890.[7] In the report of the Metropolitan Asylums Board[8] in London, where the case-mortality from diphtheria in hospitals is generally much lower than on the continent, the fatality in 1889 was 40.7 per cent; in 1890, 33.5 per cent; in 1891, 30.6 per cent; in 1892, 29.3 per cent; in 1893, 30.4 per cent. This apparent reduction in fatality since 1889 in large part disappears if only patients under fifteen years of age are considered, the corresponding percentages for these being respectively 40.7, 41.6, 36.9, 35.6, and 37.

The natural interpretation of our statistics, showing in over 7000 cases, of which at least five-sixths are from hospital practice, treated with antitoxin an extraordinary low percentage of deaths for this class of cases and showing an apparent reduction in fatality of from 50 to 60 per cent by the use of antitoxin, is that the antitoxin exerts a specific curative power over diphtheria.

What are the objections which may be and have been urged against this natural interpretation of the statistical evidence?

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[7] V. Hirsch: Archiv. f. klin. Chirurgie, Bd. 49, Heft, 4.

[8] British Medical Journal, December 29, 1894.

In the first place, it has been claimed that these observations have been made during the prevalence of unusually mild diphtheria. In some places the prevailing type of the disease seems to have been mild; but the great majority of the observers quoted in the table consider that the prevailing diphtheria in their localities has been of average severity, and they cite in many instances the simultaneous fatality of cases not treated with antitoxin as proof that the disease is not of peculiarly mild type; indeed, in several places it seems to have been of more than average severity. During the period in which Roux treated with antitoxin 300 cases in the Hôpital des Enfants Malades, with a fatality of 26 per cent; the fatality in the Hôpital Trousseau, also in Paris, and receiving a similar class of cases, was 60 per cent.

But even if it be admitted, for the sake of argument, that the prevailing type of diphtheria during the past year has been mild, it is to be considered that the influence of this milder type upon the cases received in many hospitals appears chiefly in the reduction of their number, and far less in a change in the character of the cases admitted. This is the statement of von Ranke, of Bokai, and of several other physicians in charge of diphtheria wards. They say that so far as their hospitals are concerned, as a rule, severe and advanced cases are sent there by physicians in the city, often for operation to relieve laryngeal stenosis, and that when the epidemic is mild in character they receive fewer cases, but not many milder cases. Doubtless these conditions will not hold for all hospitals, particularly not for such as are intended for the compulsory isolation of all cases of diphtheria which cannot be properly isolated at their homes, but they are probably applicable in large part to most of the hospitals from which come the reports now under consideration.

So far as I can judge, no proof has been brought forward in support of the opinion that the low percentage of fatality of diphtheria treated with antitoxin can be referred in any large measure to the prevalence of an unusually mild type of the disease, although in a few scattered groups of cases, particularly some of the smaller series in my table, this may be in part the explanation.

I attach decidedly more weight to a second criticism of antitoxin statistics which has been made, namely, that in hospitals where the serum treatment has been carried out a proportionately larger number of cases are received now than formerly in the earlier stages of diphtheria. The advocates of the treatment have properly insisted upon the importance of early injection of the serum, and, especially during the time when the serum was not to any extent in the hands of general practitioners, and it would be natural to suppose that physicians would send their patients and parents take their children to such hospitals as soon as possible after recognition of the disease. Inasmuch as with any approved method of treatment of diphtheria the results are better the earlier it is begun, it is evident that statistics based on the former experience with the treatment of diphtheria in hospitals would not be altogether comparable with the antitoxin statistics from the same hospitals.

It is, however, very difficult to say how much allowance is to be made for this criticism. There has been widespread skepticism among physicians and the general public as to the value of the treatment. Thus Rapmund, in his efforts to establish in the district of Minden a collective investigation of serum-therapy in diphtheria, found the physicians so skeptical that of 194 practitioners only twenty would use it at all, and only two employed it extensively. There are also statements as to the unwillingness of parents to have it tried on their children. Many of the reports state that during the period of serum treatment cases were not received in any earlier stages of the disease than formerly; in a few of the reports, as, for example, in that of Kuntzen from Oschersleben, it is said that physicians were induced to send their patients early in the disease. In Berlin and some other cities there has been a marked increase in the number of patients with diphtheria admitted to hospitals since the introduction of the serum treatment, and this has been without a corresponding increase in the total number of cases in the cities. Heubner in his recent address at the Congress of Internal Medicine, at Munich, admits that lighter cases of diphtheria go to the hospital now, but that this is not enough to explain the great difference in fatality. There are undoubtedly considerable differences in different hospitals as to the proportion of cases admitted in early stages of diphtheria, but in many of the hospitals where the benefits of antitoxin have been most apparent, as contrasted with the previous results, it is expressly stated that the number of mild cases admitted is no greater than formerly.

If we make all due allowance for this increase in the proportion of early cases treated in hospitals, and certainly some allowance must be made, this factor is still altogether inadequate to explain the great reduction in fatality of diphtheria treated with antitoxin. This will also be apparent later when we consider the results of treatment according to the day of the disease on which it is begun.

A third criticism, namely, that the bacteriological control of the diagnosis of diphtheria operates in favor of a low mortality in antitoxin statistics has already been fully discussed.

It is manifestly improper to compare the average mortality of thousands of cases treated in hospitals with antitoxin with exceptionally favorable results at certain periods in a few hospitals in a comparatively small number of cases without serum treatment, and still more improper, as has even been done, to make such comparison with the most favorable percentages which one can find reported from private practice or in municipal mortality statistics. Surely some consideration must be given to the previous and simultaneous results obtained from cases without serum treatment in the same hospitals from which the cases reported are derived.

We have now considered the principal objections which have been made to the natural interpretation of statistics, showing an apparently great reduction in the fatality of diphtheria by the use of antitoxin. I believe that it has been shown that, even if all possible allowance be made for such assumptions as those considered, they are still wholly inadequate to account for an apparent reduction in the deaths from diphtheria by antitoxin treatment of 50 to 60 per cent in nearly 5000 cases collected from hospitals in Germany, France, Austria, England, and America, and reported by forty different physicians, most of whom are of high reputation and large experience. These statistics seem to me to establish beyond all reasonable doubt the conclusion that antitoxin is a specific curative agent for diphtheria.

It has been contended that the only absolutely convincing proof of the curative efficacy of antitoxin is the demonstration of a marked reduction in the total number of deaths from diphtheria in a city or town in proportion to all of the cases. Municipal mortality and morbidity statistics are necessarily far less accurate than hospital statistics, and, for reasons which have been stated, the prevalence of a mild type of diphtheria will have greater influence upon municipal mortality statistics for diphtheria than upon hospital statistics. It is to be expected that when sufficient time has elapsed and the enjoyment of antitoxin in the treatment of diphtheria has become sufficiently general, the reduction in mortality by its use will be apparent in general mortality statistics. At present we have little information upon this point. The mere statement of the total number of deaths without knowledge of the morbidity and of the prevailing type of disease is, of course, not decisive for either side of the question; but so far as it goes it is interesting to learn that in Boston, during the antitoxin period (January 1 to May 1, 1895) the total fatality from diphtheria was 14 per cent, as compared with a fatality of 31 per cent during the corresponding period of previous years (Mason), and that in Carthagena, Spain, during four months of employment of antitoxin, the total number of deaths was only one-fourth the average number for the same period of time during the preceding ten years.

The only antitoxin statistics which I can find based upon such material as composes municipal fatality statistics are those of Risel and of Kurth.

Risel reports the results in all of the cases treated by antitoxin during two months in the city of Halle. They are derived from the practice of thirty physicians among the poor and rich, in the houses of the patients and in hospitals, and include mild and severe cases as they presented themselves. Of the 89 patients treated in their homes, almost without exception children not over seven years of age, 6 died, giving a fatality of 6.7 per cent; 19 of these laryngeal diphtheria, of whom 4 died. Of the 25 patients treated in hospitals, 3 died, a fatality of 12 per cent; 15 of these had laryngeal involvement, of whom 3 died. The total fatality was 7.9 per cent. No data are given for comparison with the previous or simultaneous fatality of cases not treated with serum. In only a few cases, and these in hospitals, was the clinical diagnosis confirmed by bacteriological examination.

Kurth reports the results of serum treatment in the practice of sixty physicians in Bremen, and a few outlying villages, from October 8, 1894, to January 31, 1895. A circular letter was sent from the Bacteriological Institute to every physician in the city, and apparently general cooperation on the part of the physicians and the public officials was secured. In 97 cases treated with serum the diagnosis of diphtheria was established, in the great majority of the cases by demonstration of the Loeffler bacillus controlling the previous clinical diagnosis. The total case-mortality was 10.3 per cent. The fatality of the 64 cases treated in the city (hospital and private practice) was 7.8 per cent; that of 33 cases derived from the surrounding country district was 15.2 per cent; that of 35 cases treated in the city hospitals was 14.3 per cent. Laryngeal diphtheria occurred in 66 per cent of the cases in the country districts and in only 36 per cent of the cases in the city. This and the generally less favorable results in country practice are attributed by Kurth, not to greater severity of the epidemic in the former, but to the custom in the country of not calling a physician until the symptoms are urgent, and to the greater distance which physicians have to travel. If the bacteriological control of the diagnosis be disregarded—that is, if all the cases diagnosed clinically as diphtheria and treated with serum be considered—the fatality was 9.4 per cent; another illustration that bacteriological control of the clinical diagnosis results in higher, not as some have claimed in lower, percentage of deaths. Of the 50 cases of clinical diphtheria, all of the cases being included which did not show Loeffler bacilli, the fatality was only 6 per cent. During the serum period there occurred 25 cases of diphtheria not treated with antitoxin, with a fatality of 24 per cent. During the same period of the year in which the serum-treated cases occurred there were during the preceding year 148 cases of diphtheria, with a fatality of 32 per cent.

It must be conceded that these interesting reports of Risel and of Kurth speak strongly in favor of the possibility of bringing about a great reduction in the general fatality from diphtheria in cities by treatment with antitoxin. As a larger proportion of the cases in private practice can be treated in early stages of the disease than in hospitals, this reduction should be greater than that already shown by hospital statistics.

A most convincing demonstration of the healing power of antitoxin is furnished by the experience of Baginsky during an involuntary pause in the serum treatment caused by failure in the supply of serum. Between March 15, 1894, and March 15, 1895, there were treated in Baginsky's service by antitoxin 525 children, with a fatality of 15.6 per cent During the period of forced interruption of the serum treatment, this period being chiefly the months of August and September, 126 children were treated without antitoxin, with a fatality of 48.4 per cent. There was absolutely no selection of cases in either group. In his comments upon this experience Baginsky says:

> "It is all the more remarkable as the ratio of mortality of those treated with the serum, both before and after the period of interruption, varied within very small percentage figures. If one will permit figures to speak at all, there has scarcely been made on human beings a more demonstrative test of the curative power of a therapeutic agent. It was an experiment forced upon us, but it proved to us how terrible was the form of disease which we were treating, and how numerous would have been the victims without the use of the healing serum."

A similar experience has been reported by several other writers. Thus Körte noted a rise in fatality from 33.1 per cent during the serum period to 53.8 during the period of failure in the supply of serum; Ganghofner, under similar conditions, a rise from 12.7 per cent to 53.2 per cent; Heim, from 22 per cent to 65.6 per cent, and during the epidemic in Trieste the fatality rose from 18.7 per cent to 50 per cent, when the serum failed. All of these highly significant observations were made on cases occurring in the same epidemic, the period of enforced interruption of the serum treatment being preceded and followed by the periods of serum treatment.

We have considered thus far mainly the hospital statistics. These are, for manifest reasons, more numerous, larger, and more carefully analyzed than those from private practice. It is, however, in private practice, especially among those classes who are in the habit of calling a physician early in the disease, that the best results from serum treatment are to be expected, for here there is more frequent opportunity for timely treatment. A glance at Table I will show that in general the fatality of diphtheria treated with serum in private practice is much lower than in hospitals.

If we summarize the 18 reports from private practice in Table I, we have 663 cases of this class treated with antitoxin and among these are 46 deaths, giving a fatality percentage of only 6.9. This would indicate that the serum treatment may reduce the fatality from diphtheria in private practice to nearly one-third that under the same treatment in hospitals. Some of the reports of the results of serum treatment in private practice furnish, indeed, most remarkable evidence of the efficacy of this treatment.

Most of the reports attempt some sort of classification of the cases treated with antitoxin. The simplest and most common, although not the most valuable, is the division according to degrees of apparent severity, expressed by such epithets as mild, moderate, severe, very severe. Such a classification is, of course, only of limited value, as even the mildest case of diphtheria may unexpectedly assume a malignant character. If, as we believe to be proved, antitoxin injected in time arrests the local process and the constitutional disturbance, then many of the cases which appear under the head of mild cases in antitoxin statistics would, under other methods of treatment, have become severe cases, and would be so recorded. Indeed, with the early administration of antitoxin there should be comparatively few severe cases.

The classification of diphtheria adopted by Roux into angina and croup with and without microbic association has been followed by some of his successors. So far as this classification is actually based upon a correct separation of pure diphtheria from diphtheria with mixed infection, it is of the utmost importance in determining the relative value of antitoxin in the treatment of these two divisions of diphtheria. I have already expressed the opinion, however, that the bacteriological examination of the exudate and secretions in the throat is not decisive in determining the presence or absence of mixed infection. Still Roux's analysis of his cases on this basis indicated clearly that the serum was far more efficacious in diphtheric anginas and croup which yielded pure cultures of the Loeffler bacillus than in those which gave in addition to the Loeffler bacillus cultures of other pathogenic bacteria. In presenting these results the epithet "pure" applied to angina or croup is to be understood to signify that only the Loeffler bacillus was found in the cultures, and the epithet "associated" to signify that this bacillus was found in cultural association with the coccus of Brisou, staphylococci or streptococci, most commonly the last. The "corrected" percentages are those obtained after subtracting deaths occurring within twenty-four hours after admission.

Of the 300 cases treated with serum with a total fatality of 26 per cent reported by Roux, Martin, and Chaillou, there were 120 pure anginas, fatality 7.5 per cent (corrected 1.7 per cent); 49 associated anginas, fatality 24.2 per cent (corrected 17.7 per cent); of not operated croup, 4 pure, fatality 0; 6 associated, fatality 16.6 per cent; of operated croup, 49 pure, fatality 30.9 per cent (corrected 24.4 per cent), and 72 associated, fatality 56.9 per cent (corrected 43.1 per cent).

Of Moizard and Perregaux's 231 cases, total fatality 14.7 per cent, there were 44 pure anginas, fatality 4.5 per cent; 42 associated anginas, fatality 14.3 per cent; 94 pure croup, fatality 18.5 per cent; 51 associated croup, fatality 17.6 per cent. No correction is made in these percentages.

Of Sevestre and Meslay's 150 cases, total fatality 10 per cent, there were 29 pure anginas, fatality 3.4 per cent; 24 associated anginas, fatality 12.5 per cent (corrected 8.3 per cent); 67 pure croup, fatality 8.9 per cent (corrected 7.5 per cent); 30 associated croup, fatality 16.6 per cent (corrected 6.6 per cent).

It will be observed that in the last two reports the excess in fatality in the "associated" diphtherias is much less striking than Roux's statistics, and in some cases disappears altogether. This I am inclined to attribute to failure of the methods employed to indicate properly the division into pure and mixed infections, for the testimony is unanimous that the serum is of far less benefit in mixed diphtheria than in uncomplicated diphtheria, the most common and dangerous complicating microorganism being *Streptococcus pyogenes.*

Most noteworthy has been the improvement in the results of serum therapy of diphtheria in the Paris hospitals since Roux's original communication to the Congress in Buda-Pest in September, 1894. The fatality has descended from Roux's original percentage of 26, in the later reports, to 14.7, 12, and 10 per cent, and, according to a recent statement of Moizard and Bouchard (July, 1895), it at present oscillates between 8 and 14 per cent. These are the best results which have hitherto been reported from any hospital for any large number of cases, and they are certainly most significant. As Moizard and Bouchard in their recent communication say, "This result can no longer be attributed to fortunate series of cases, as was claimed at the beginning by adversaries of the method. Thousands of patients have been treated, and it can now be said that the controversy is closed." This striking descent from Roux's first figures is not, however, attributed by the writers wholly to improvements in the methods of serum-therapy. As Roux pointed out in his first paper, the hygienic conditions in the two Paris hospitals from which these statistics come were very bad. These conditions have since then been greatly improved, and this reform has been especially manifest in the reduction of the deaths from bronchopneumonia.

A most important classification of diphtheria for estimating the curative value of antitoxic serum is that into cases without and with laryngeal stenosis, and especially when such degrees of stenosis are considered as require operative interference by tracheotomy or intubation. I have therefore prepared the following table (Table II), which gives the results of antitoxin treatment in operated and nonoperated cases of diphtheria. This, of course, is not equivalent to a division into anginas and croup, as many cases of croup are included in the nonoperated cases; but I have desired to submit the new method of treatment to the most severe test. No one can claim that laryngeal diphtheria requiring intubation or tracheotomy is anything but a severe disease. If the benefits of antitoxin are unmistakably manifested in these operated cases of croup, then the test is an *experimentum crucis,* and puts an end to the objections of those who assert that the apparently favorable results of serum-therapy in diphtheria are attributable mainly to the large proportion of mild cases treated.

The same reports with a few additional ones, for which references are given, have been used for Table II as for Table I, but many of the reports in Table I were not available for this table, as the writers did not always present their results in a form which fitted into the classification adopted. The table gives for each report the total number and fatality of cases treated, as in Table I, the number and fatality of cases not operated on (including cases of croup), the number and fatality of cases operated on, "T" signifying tracheotomy, "I" intubation, "I and T" signifying intubation followed by tracheotomy, and, so far as reported, the previous or simultaneous percentages of fatality from operation in cases treated without antitoxin. In the final column are pertinent statements concerning the cases in the series. Some reports are inserted which do not give the number of cases under the different headings. These, of course, cannot be used in the summary giving the totals.

In 41 reports there are 648 tracheotomies with 258 deaths, a fatality of 39.8 per cent; 342 intubations with 99 deaths, a fatality of 28.9 per cent, and 26 intubations followed by tracheotomy with 14 deaths, a fatality of 53.8 per cent.

There are 211 operated cases in which it is not stated how many are tracheotomy or intubation. These gave a fatality of 40.2 per cent. They were probably for the most part tracheotomies.

The reports giving the previous or simultaneous fatality from tracheotomy contain 510 cases of tracheotomy, with 217 deaths, or 42.5 per cent. If the fatality of these cases be reckoned on the basis of the preceding or simultaneous fatality, selecting the lowest figures given, there would have been 329 deaths, or 64.5 per cent. There was, therefore, an apparent reduction in fatality of 34.1 per cent by the serum treatment.

Making a similar estimate on the basis of previous fatality from intubation, there were 250 intubations, with 79 deaths, or 31.6 per cent, instead of 156 deaths, or 62.4 per cent. There was, therefore, an apparent reduction

TABLE II

Fatality in Operated and Not-operated Cases of Diphtheria Treated with Antitoxin

T, signifies tracheotomy; I, intubation; I and T, intubation followed by tracheotomy

Reporter	Total cases	Deaths	Not operated cases	Deaths	Operated cases	Deaths	Previous fatality from operation	Remarks
Roux, Martin, and Chaillou.	300	78 (26 p. ct.) ..	179	22 (12.8 p. ct.)	121 T	56 (46 p. ct.)....	67 p. ct......	Deducting those which died in less than 24 hours after admission to the hospital, there remain 107 tracheotomies with 42 deaths (39.2 per cent.)
Kossel.........	117	13 (11.1 p. ct.)	94	1 (1.1 p. ct.).	23 T	12 (52.2 p. ct.)..	....	In all of the fatal operations it was necessary to perform tracheotomy within 12 hours after admission.
Körte..........	121	40 (33.1 p. ct.)	79	18 (22.8 p. ct.)	42 T	22 (52 4 p. ct.)..	77.5 p. ct....	Of 8 tracheotomized cases under 2 years of age, 5 died (62.5 per cent). The previous fatality for tracheotomy under 2 years was 90.7 per cent.
Sonnenburg....	95	16 (16.8 p. ct.)	61	8 (13.1 p. ct.)	34 T	8 (23.5 p. ct.)..	38 p. ct......	The fatality of 38 per cent in the table is that of 47 tracheotomies performed during the period in which the supply of antitoxin temporarily was exhausted and could not at once be replaced.
Baginsky......	525	83 (15.6 p. ct.)	430	47 (10.9 p. ct.)	95 { 53 T 54 I (in 12 cases tracheotomy followed intubation).	36 { 34 T 2 I } 37.8 p.c. (of the secondary tracheotomies 12 died). (37.9 per cent.)	64.4 p. ct. T 44.8 p. ct. I	During two months in which the supply of serum failed the general fatality rose to 48.4 per cent, and that from tracheotomy and intubation to 62.2 per cent. The fatality at once fell upon reintroduction of the antitoxin treatment.
v. Widerhofer..	300	71 (23.7 p. ct.)	192	20 (10.4 p. ct.)	108 T or I	51 (47.2 p. ct.)..	....	22 cases of laryngeal diphtheria (croup) recovered without operation.
v. Ranke.......	96	19 (19.7 p. ct.)	54	6 (11.1 p. ct.)	42 I	13 (30.9 p. ct.)..	61–75 p. ct...	21 cases of croup recovered without operation.
Stintzing......	59	12 (20.1 p. ct.)	43	5 (11.6 p. ct.)	16 T	7 (44 p. ct.)....	49 p. ct......	
Schröder.......	63	8 (12.7 p. ct.)	32	5 (15.6 p. ct.)	31 T	3 (9.67 p. ct.)..		
Vierordt.......	63	16 (25 p. ct.)..	48	9 (18.75 p. ct.)	15 T	7 (46 p. ct.)....	....	Deducting 8 cases in a hopeless condition on admission, there was only one death among the non-tracheotomized cases, a death-rate of only 2.7 per cent.
Rumpf.........	22	2 (8 p. ct.)...	15	0	7 T	2 (28.6 p. ct.)..		
Lebreton and Magdelaine.	258	31 (12 p. ct.)..	183	8 (4.4 p. ct.).	75 { 24 T 51 I	23 { 9 T (37.5 p.c.) 14 I (27.5 p.c.)	73.2 p. ct. T	Of the intubated cases 7 died in less than 24 hours after admission. Deducting these, the fatality from intubation was only 18.1 per cent.
Le Gendre, Moizard and Perregaux. Sevestre and Meslay.	398	52 (13.1 p. ct.)	316	23 (7.6 p. ct.)	82 { 51 T 21 I 10 I & T	29 { 20 T (39.2 p.c.) 5 I (23.8 p.c.) 4 I&T (40 p.c.) (35.4 per ct.)	86 p. ct. T	Deducting 15 deaths in less than 24 hours after admission, the total fatality is reduced to 9.7 per cent. These cases are those treated in the Hôpital Trousseau, in Paris, from the middle of September to December 25, 1894.

TABLE II—*Continued*

Reporter	Total cases	Deaths	Not operated cases	Deaths	Operated cases	Deaths	Previous fatality from operation	Remarks
Ganghofner	110	14 (12.7 p. ct.)	66	8 (12 p. ct.)	44 I or T	6 (13.6 p. ct.)	59.8–78 p. ct.	12 laryngeal diphtherias recovered without operation. During period in which the supply of serum failed the general fatality rose to 53.2 per cent, and that of operated cases to 68.9 per cent.
Soltman	89	13 (14.6 p. ct.)	48	2 (4.2 p. ct.)	41 I	11 (27 p. ct.)	68 p. ct.	
Bokal	120	31 (25.5 p. ct.)	71	10 (14 p. ct.)	49 I	21 (43 p. ct.)	70 p. ct.	In 14 cases presenting symptoms of moderate laryngeal stenosis upon admission these symptoms disappeared after injection of antitoxin without operation.
Hein	27	6 (22 p. ct.)	21	4 (19 p. ct.)	6 { 2 T 4 I	2 { 1 T (50 p. ct.) 1 I (25 p. ct.) 33.3 per ct.	....	During period when supply of serum failed the general fatality rose to 65.6 per cent. Hein treated altogether 48 cases in two groups, but of his second group of 21 cases, 12 were still under treatment at date of report, and these are not included in my table.
Börger	30	2 (6.66 p. ct.)	25	1 (4 p. ct.)	5 T	1 (20 p. ct.)	....	313 cases of diphtheria treated in the same hospital (Greifswald) from Oct. 1893, to Sept. 1894, gave a fatality of only 14.5 per cent.
Moeller	76	27 (35.5 p. ct.)	28	8 (28.6 p. ct.)	48 T	19 (39.6 p. ct.)	55.6 p. ct.	
Kuntzen	25	3 (12 p. ct.)	20	1 (5 p. ct.)	5 T	2 (40 p. ct.)		
Charon	13	4 (30.8 p. ct.)	2	0	11 T	4 (36.4 p. ct.)		
Washbourn, Goodall, and Card.	72	14 (19.4 p. ct.)	63	11 (17.5 p. ct.)	9 T	3 (33.3 p. ct.)	91.7 p. ct.	In 13 previous series of 9 tracheotomies in each group the average of deaths numbered 8¼.
Herringham	18	3 (16.7 p. ct.)	7	0	11 { 10 T 1 I	3 (30 p. ct.) T	65 p. ct. T	The 7 unoperated cases were mild.
Germonig (Civic Hosp. of Trieste.)	224	42 (18.7 p. ct.)	178	18 (10 p. ct.)	46 { 2 T 44 I	24 I (54.5 p. ct.)	85.5 p. ct. T. 75 p. ct. I	During a period of exhaustion of the supply of serum the general fatality rose to 50 per cent. These data are from abstracts in the British Med. Journ., Feb. 2, 1895, and the Deutsche med. Wochenschrift, 1894, No. 52. The general serum fatality is variously given as 18.7, 20.3, and 22 per cent.
Witthauer	36	5 (14 p. ct.)	16	1 (6.25 p. ct.)	20 T	4 (20 p. ct.)	25 p. ct.	Only one adult in this series.
Rapmund	100	7 (7 p. ct.)	98	7 (7.1 p. ct.)	2 T	0	....	Collection of cases treated in private practice by several physicians in Minden.
Heidenhain	24	3 (12.5 p. ct.)	22	2 (9.1 p. ct.)	2 T	1 (50 p. ct.)		
Risel	114	9 (7.9 p. ct.)	95	5 (5.3 p. ct.)	9 T	4 (21 p. ct.)		
v. Muralt	58	2 (3.4 p. ct.)	38	0	20 { 14 I 2 T 4 I & T	2 (10 p. ct.)	....	After intubation (14 cases) no deaths; after intubation and tracheotomy one death; after tracheotomy one death.

TABLE II—*Continued*

Reporter	Total cases	Deaths	Not operated cases	Deaths	Operated cases	Deaths	Previous fatality from operation	Remarks
D'Espine	60	6 (10 p. ct.)	49	2 (8.2 p. ct.)	11 T	4 (36.4 p. ct.)	....	10 cases of croup recovered without operation.
v. Engel	39	10 (25.5 p. ct.)	30	7 (23.3 p. ct.)	9 T	3 (33.3 p. ct.)	85 p. ct.	The fatality of 42 unoperated cases not treated with antitoxin was 33.4 per cent, that of 20 tracheotomized cases not so treated 85 per cent.
Codd & Whitehouse.	11	4 (36.4 p. ct.)	7	2 (28.6 p. ct.)	4 { 3 T 1 I	0 I 2 T (66.6 p. ct.) 50 p. ct. of operated cases.	....	Three of the four deaths were from croup.
Winkfield	22	4 (18.2 p. ct.)	19	2 (10.5 p. ct.)	3 T	2 (6.66 p. ct.)	85.7 p. ct.	
Sigel	100	12 (12 p. ct.)	No. of cases not given	....	No. of cases not given.	(20.3 p. ct.) T	63.3–70 p. ct.	The fatality of tracheotomized cases in 1894, up to the date of beginning treatment (Oct. 4) was 70 per cent; after treatment with antitoxin 20.3 per cent.
Galatti	No. of cases not given	....	No. of cases not given	....	15 I	6 (40 p. ct.)	52.6 p. ct. I.	Quoted in Le Bulletin Médical, 1895, p. 364.
Kraske	Not given	....	......	....	16 T	5 (31.25 p. ct.)	28 p. ct. (in 12 cases).	Says that he has tracheotomized 12 cases not treated with antitoxin with a fatality of only 38 per cent. Abst. in Münchener med. Wochenschrift, 1894, p. 1062.
Bonain	Not given	....	......	....	5 I	2 (40 p. ct.)	60.5 p. ct.	Le Bulletin Médical, April 17, 1895.
Van Nes	52	12 (23 p. ct.)	30	4 (13.3 p. ct.)	22 { 15 T 7 I	8 (36 p. ct.) I & T	48–73 p. ct.	During ten preceding years the lowest fatality of operated cases was 52 per cent, not operated 16 per cent. In three groups of 52 cases each, preceding the introduction of antitoxin treatment, the lowest fatality of operated cases was 48 per cent, of not operated cases 19 per cent.
Leichtenstern & Wendelstadt.	123	25 (20.3 p. ct.)	86	9 (10.4 p. ct.)	37 T	16 (43.2 p. ct.)	64 p. ct.	Of 17 tracheotomized cases less than 2 years old, 9 died; between 2 and 4 years, 4 out of 11 died, of 13 unoperated cases under 2 years, 2 died; of 21 between 2 and 4 years, 2 died.
Kohts	39	Not given	Not given	(9.1 p. ct.)	Not given.	30.77 p. ct. T	....	Abst. Münchener med. Wochenschrift, June 11, 1895.
Timmer	31	6 (19.4 p. ct.)	25	5 (20 p. ct.)	6 T or I	1 (16.6 p. ct.)		
Mason	306	81 (26.4 p. ct.)	275	62 (22.5 p. ct.)	31	19 (61 p. ct.)	90 p. ct. ?	The statement is that the fatality from tracheotomy was reduced by the antitoxin about one-third.
Kurth	97	10 (10.3 p. ct.)	82	7 (8.5 p. ct.)	15 T	3 (20 p. ct.)	....	Of 32 cases of laryngeal diphtheria not operated upon, 30 recovered.
Total of 38 reports.	4294	784 (18.3 p. ct.)	3127	350 (11.2 p. ct.)	1167	434 (37.2 p. ct.)	....	Of the total cases 27.2 per cent were operated upon by tracheotomy or intubation.

in the fatality of intubated cases of 49.5 per cent, as the result of serum treatment.

The fatality of 3127 nonoperated cases was only 11.4 per cent.

Of the 4294 cases in Table II, 27.2 per cent required tracheotomy or intubation. There were, however, many more cases of laryngeal diphtheria in this group than the ratio of operative cases would indicate, for it is the testimony of the great majority of the observers that the stenotic symptoms of laryngotracheal diphtheria are relieved without the necessity of operation in a much larger proportion of the cases treated with antitoxin than by any other method of treatment. As is well known, recovery without intubation or tracheotomy from descending laryngotracheal diphtheria, especially in children, is exceptional under all other methods of treatment, and in the greater relative frequency with which such recovery occurs under serum treatment is a strong proof of the efficacy of antitoxin.

Of Kossel's 44 cases of laryngeal diphtheria treated with antitoxin, 21 (47.7 per cent) recovered without operation; of von Widerhofer's 130 stenotic cases treated with serum, 22 (16.9 per cent) recovered without operation; of von Ranke's 63 cases, 21 (33.3 per cent); of Vierordt's 24 cases, 9 (37.5 per cent); of Ganghofner's 56 cases, 12 (21.4 per cent); of Bokai's 63 cases, 14 (22.2 per cent); of d'Espine's 21 cases, 10 (47.6 per cent). Von Ranke says that before the use of serum at most 5 per cent of his cases of laryngeal stenosis escaped operation, whereas now 33 per cent escape. Of Ganghofner's stenotic cases formerly 12 per cent escaped operation, whereas now 21 per cent escape. The experience of Heubner and many others is similar.

In this respect, as in so many others, the results in the Paris hospitals have been most favorable. Of Moizard and Perregaux's 145 cases of croup, 90 (62.1 per cent) recovered without intubation or tracheotomy. Roux, Martin, and Chaillou say:

> "Of 169 children, admitted to the service for diphtheric angina, 56 presented laryngeal symptoms; 31 had hoarse voice, and in 25 the voice was so far extinguished and the dyspnoea (tirage) so marked that one might believe that the latter patients should be operated on. Under the influence of the serum (and in these cases one should not fear to make an injection every twelve hours) the dyspnoea diminished, then occurred only paroxysmally, the child coughed up false membrane, and at the end of two or three days the respiration became normal to the great astonishment of the internes and personnel of the pavilion who, with their large experience of children affected with croup, indeed, thought that operation could not be avoided. Today, in the presence of a child with dyspnoea, it is not necessary to press for operation. One can inject the serum and wait as long as possible. Since the introduction of the serum the number of tracheotomies in the pavilion has diminished."

Out of his large experience Baginsky expresses himself in these vigorous words:

> "Here, again, the observation of the individual cases of laryngeal stenosis, and more especially of those which do not come to the point of operation, speak to me more forcibly than the statistical figures. The surprising regression of the laryngostenotic respiratory phenomena, the freedom of breathing, the disappearance of the hoarse voice and the croupy cough, the euphoria of the children, the change in their general condition, so that two days after the injection they are sitting up in bed, playing and contented and observant of their surroundings; all of these produce in him who has had before his eyes for years the hopeless picture of continually progressing laryngeal stenosis, in very truth, ineffaceable impressions."

Experience based upon such a large number of cases and careful clinical observation must be regarded as representing the norm. That there may be deviations from this norm, even in a fair number of cases, seems to be illustrated by the experience of Leichtenstein and Wendelstadt, who in 123 cases of diphtheria with 37 tracheotomies, were not able to note any material reduction in the proportion of cases requiring tracheotomy as compared with former series of cases. Their observations were uncontrolled by bacteriological diagnoses.

Another point to be considered in this connection is of capital importance as an indication of the value of serum treatment. Cases which are free from symptoms of laryngeal involvement at the time of injection of the serum do not develop such symptoms later, or do so only very exceptionally, unless evidences of such involvement appear within twenty-four hours after the injection.

Regarding neither this nor any other point is there entire unanimity of opinion in the various reports, nor is such to be expected from observers of limited numbers of cases with unequal distribution in the various groups of mild cases, of early cases, of anginas, of croup, of pure diphtheria, of septic diphtheria, etc., to say nothing of the absence in some reports of any bacteriological control of the diagnosis and of treatment by insufficient doses or inferior quality of serum. I am only surprised that the conflicting statements are not more numerous. But there are not many points concerning which there are so few differences of statement as concerning the efficacy of antitoxin in preventing descent of the diphtheritic process to the larynx and the trachea. Over and again one can read in the reports such statements as that in all of the patients who entered without laryngeal diphtheria the larynx remained free, or that unless the symptoms of stenosis appeared within the first twenty-four hours after injection of the serum they were not observed at all, or only most exceptionally. Among the many vouchers for these statements may be cited Kossel, Roux, Baginsky, von Widerhofer, Heubner, von Ranke, Vierordt, Ganghofner, Escherich, Bokai, Van Nes, Kurth.

It is this power of antitoxin to check the spread of the diphtheritic process from the tonsils and pharynx into the larynx, and from the larynx into the bronchi, which has impressed many observers in favor of the new treatment more forcibly than any other feature of their experience with its action. Thus Vierordt observed that of 24 children with diphtheria who were admitted with unaffected larynx, and treated with antitoxin, only one developed temporarily a hoarse cough on the third day. In all of the others the larynx remained free. Of 23 patients who were admitted with unaffected larynx not long before the introduction of the serum treatment, nine afterward developed croup. This is doubtless a somewhat unusual experience as regards the large proportion of cases of croup developing under previous methods of treatment.

It follows from what has been said that the ratio of operative cases in antitoxin statistics will in general be smaller than in statistics of cases of the same character treated by other methods. On the one hand there will be fewer laryngeal stenoses developing after commencement of the treatment, and on the other hand a larger number of recoveries from laryngeal diphtheria without the necessity of operation.

The following figures serve to illustrate this point. In the service from which the cases reported by Roux were derived tracheotomy was performed before the serum period in 50 per cent of the cases of diphtheria, after the introduction of serum in 40 per cent. The later Paris reports give a much greater reduction in the ratio of tracheotomies. In Baginsky's service 43.9 per cent of the cases were operated on before the use of serum, and 18.1 per cent after its introduction; in von Ranke's service the corresponding figures are 57 per cent before and 43.5 per cent after; in Bokai's, 65.6 per cent before and 40.8 per cent after. As already mentioned, Leichtenstern's figures, 32 per cent before and 30 per cent after serum, are exceptional.

It is furthermore to be considered that in view of the power of antitoxin to abate beginning and moderate symptoms of stenosis, operation will be delayed rather than hastened, and, when performed, the indications for it will generally be urgent. For manifest reasons, most of the operations will fall within a period not remote from the time of injection of the serum. Of the 121 tracheotomies in the report of Roux, Martin, and Chaillou, 102 were performed either before the first injection of antitoxin, or within twelve hours afterward; 14 between the twelfth and the thirty-sixth hour after inception of the serum treatment, and only 5 later than thirty-six hours after the injection of the serum. Of the 23 tracheotomies with 12 deaths reported by Kossel the operation was performed within the first twelve hours in all of the fatal cases, and of the 11 successful cases it was performed in 9 on the day of admission to the hospital, in 1 on the second day, and in 1 on the following day. Kossel refers the increase in the stenotic symptoms after injection of the serum in the last two cases to the separation of the false membranes, a point to which others have also called attention as an effect of antitoxin, and which is to be borne in mind in cases of croup treated by antitoxin.

Turning now to the results of tracheotomy and intubation in cases treated with antitoxin, we find in Table II that in 41 reports there were 648 tracheotomies with 258 deaths, a fatality of 39.8 per cent, and 342 intubations with 99 deaths, a fatality of 28.9 per cent, and 26 intubations followed by tracheotomy, with 14 deaths, a fatality of 53.8 per cent. These are not unheard-of fatalities from these operations, but they are so low as to indicate decidely remedial action of antitoxin

The percentage of fatality from tracheotomy in diphtheria given by Monti from a total of 12, 736 cases up to 1887 is 73.3. The percentage given by V. Hirsch in 1654 tracheotomies in diphtheria in von Bergman's clinic in Berlin during the last ten years and seven months (up to July 31, 1894), is 68.7 per cent, the fatality during the first four years of this period being 70.5 per cent, and during the last four years 63.8 per cent. The fatality during the first year of life was 98.8 per cent, and sank for each year to the ninth, when it was 41.7 per cent, and after the tenth year it rose again.

More proper, however, than comparison with these latter percentages is comparison with the percentages of fatality in the same hospital or place from which the respective groups of cases are reported. It will be observed that with one exception in the table the percentage of deaths following operation in cases treated by antitoxin is lower, and generally very much lower, than the previous or simultaneous fatality. Kraske's exceptional series is of so few cases (only 5 with and 12 without serum) as to be without any significance. The lowest fatality thus far reported in a series is 3 deaths in 31 tracheotomies with serum treatment, or a fatality of only 9.67 per cent. This is reported by Schroeder from the hospital in Altona.

If for each group of cases we estimate the number of deaths which would have occurred in the tracheotomized cases treated with serum, on the assumption that the previous or simultaneous fatality in cases not treated with serum had obtained, we obtain the following result: The actual percentage of deaths in 510 tracheotomized cases treated with serum was 42.5. The percentage of fatality in these cases, estimated on the basis of previous or simultaneous fatality in the same hospitals, would be 64.5. There was, therefore, an apparent reduction in fatality by the serum treatment of 34.1 per cent. This difference between actual and estimated fatality is greater than is observed in any ordinary experience of variations in fatality during a series of years in the same hospital from tracheotomy in diphtheria.

I confess to some surprise that the analysis of the tracheotomized cases treated by serum should have yielded results so strikingly favorable to antitoxin treatment. When one considers that the benefits of serum treatment are most strikingly apparent when the treatment is begun early in the disease, and become more and more doubtful after the third day, it would have been a convincing argument against the treatment if these benefits were not conspicuously manifest in cases of diphtheria requiring tracheotomy, for, as has been explained, the great majority of these tracheotomized cases are already the subject of advanced laryngeal stenosis when the antitoxin is first injected. There are, however, not a few cases which begin apparently as laryngeal diphtheria (croup d'emblée), or in which the involvent of the larynx occurs within twenty-four or forty-eight hours after the onset of the attack. That careful observation would reveal in many of these apparently primary or early laryngeal diphtherias a latent or slightly manifested diphtheric angina, I believe to be true.

It is interesting to note that in several reports the benefit of serum treatment has been much more evident in the operated cases than in those not operated on, although this is not the rule. Indeed, Leichtenstein and Wendelstadt find in their series of 123 cases that the difference in favor of the serum in their non-operated cases was so small as to be without significance, whereas there was a difference in favor of the serum of 20.8 per cent in their tracheotomized cases, with and without serum treatment. They attribute, therefore, the entire benefit of the serum, in their experience, to its action in tracheotomized cases. Their experience, however, is exceptional, although in a measure approached by that of Ganghofner and of Van Nes. On the other hand, in Vierordt's experience the entire benefit of antitoxin seemed to be in the nonoperated cases. As has been repeatedly explained, such diversities of experience with limited numbers of cases are to be expected, and the norm can be established only by observations of large numbers of cases in different places and at different times. This norm is that both operated and nonoperated cases are benefited by antitoxin, and that the difference in each class between serum fatality from other methods of treatment is a large one.

The fatality of intubated cases in Table II treated with antitoxin is 28.9 per cent, which is 10.9 per cent less than the fatality of tracheotomized cases.

Before the introduction of the serum treatment a collective investigation was set on foot by the German Gesellschaft für Kinderheilkunde to determine the average fatality following intubation. In 1893 von Ranke reported to the Society that 1445 cases of diphtheria with laryngeal stenosis treated by intubation gave a mortality of 62.5 per cent. This result was interpreted in favor of intubation as opposed to tracheotomy. There is a difference of 33.6 per cent between this percentage and that obtained from our 342 intubated cases treated with antitoxin. This difference is so great that, after making all due allowance for possible differences in the series of cases entering into the two groups of statistics, it seems impossible to explain it otherwise than as a powerful additional support of the arguments already presented in support of the claims of antitoxin. Here, certainly, the objection that the cases treated by antitoxin were light ones cannot be made.

Table II enables us to compare the fatality of 250 intubated cases treated with antitoxin with the fatality estimated on the assumption that the previous or simultaneous fatality from intubation in the same hospital had obtained in the several groups. By this calculation we find the actual fatality to be 31.6 per cent, and the estimated fatality 62.4 per cent. In other words, there was an apparent reduction in the fatality of intubated cases of 49.5 per cent, as the result of the serum treatment.

However distrustful one may be of statistical evidence in therapeutics—and previous experience justifies much distrust—I fail to see on what credible assumption this striking reduction of fatality can be explained otherwise than as demonstrative of the specific curative power of antitoxin in diphtheria.

Lamentable for the victims, but adapted to convince the skeptical, were the experiences of Baginsky and of Ganghofner during the periods of failure in the supply of serum. During the enforced two months' interruption of the serum treatment (August and September) in Baginsky's service there were 116 cases of laryngostenosis with a fatality of 62.2 per cent, as opposed to a fatality of 37.8 per cent in the serum periods which preceded and followed the pause. The percentage of operations rose to 55.2, as opposed to 18.1 per cent during the periods of serum treatment, and this without any change in the general character of the cases admitted. During the serum periods there were more intubations than tracheotomies, whereas during the pause there were 45 tracheotomies and 19 intubations, 13 of the latter requiring secondary tracheotomy. In Ganghofner's service the fatality of the operated cases rose from 13.6 per cent to 68.9 per cent during the interruption in the supply of serum.

There remain two points to be touched upon before dismissing the laryngeal stenoses. These are the substitution of intubation for tracheotomy in a larger and larger proportion of the laryngeal diphtherias requiring operative interference and treated by the serum, and the shortening of the period during which the tube or the tracheal canula is required to be kept in the air passage.

An agent which would arrest the progressive descent of the diphtheritic process from the larynx into the bronchi and hasten the disappearance of the obstructive exudate is just what was needed to make the intubation the ideal operation for the relief of the great majority of cases of croup requiring operative interference. Such an agent we now possess in antitoxin for a large group of cases, and we are not surprised, therefore, to find that the employment of intubation, as a substitute for tracheotomy, has been greatly extended by the introduction of serum therapy.

Several writers give figures showing that serum therapy materially hastens the time when extubation or removal of the tracheal canula is permissible, but I have not attempted to collect these figures.

Of the 3127 nonoperated cases, including, as already stated, many cases of croup, 350 died, giving a fatality of 11.2 per cent. In V. Hirsch's statistics of diphtheria from von Bergmann's clinic for ten years the average fatality of nonoperated cases (1004) was 26 per cent, varying only from 25.9 per cent during the first four years of the period to 27.3 per cent during the last four years. There is, however, no general standard of fatality for cases of diphtheria not operated on. The variations are within very wide limits, as might be expected. Only a comparatively small number of the reports give separately the previous or simultaneous fatality of nonoperated cases not treated with serum. I find in the reports the following data on this point. In Roux, Martin, and Chaillou's report the previous fatality of nonoperated cases averaged 33.9 per cent, the minimum being 32.1 per cent and the maximum 47.3 per cent, as opposed to 12.8 per cent under the serum treatment; in Baginsky's report the corresponding figures are 31.6 per cent *versus* 10.9 per cent; in Bokai's, 34.5 per cent *versus* 14 per cent; in Ganghofner's, 15.8 per cent (the lowest in a series of years) *versus* 12 per cent; in Van Nes's, 33 per cent, the average of ten years, with a minimum of 16 per cent and a maximum of 41 per cent, *versus* 13.3 per cent; in Leichtenstern and Wendelstadt's, 15 per cent *versus* 10.4 per cent.

Age is a factor of such prime importance in the prognosis of diphtheria that I have prepared the following table (Table III), in which the cases treated with serum, collected from twenty-five reports, are classified according to age. Unfortunately, there is very little uniformity of system in the different reports in giving the results according to the ages of the patients, many of the reports simply stating the number of adults or the maximum age

TABLE III

AGES OF PATIENTS TREATED WITH ANTITOXIN

In each space the upper number is that of the cases, and the lower that of the deaths

Reporter	Total cases	Und. 1 yr.	1–2 years	2–3 years	3–4 years	4–5 years	5–6 years	6–7 years	7–8 years	8–9 years	9–10 years	10–12 years	12–15 years	Over 15 yrs	Remarks
Kossel	117 13 (11 p. ct.)	3 1	4 0	18 2	14 3	20 3	10 2	11 0	10 0	7 1	7 0	12 0	1 1		
Körte	121 40 (33.1 p. ct.)	..	..	..	..	..	..	..	..	..	..	..	..	..	Of 15 infants under 2 years, 8 recovered and 7 died.
Baginsky	525 83 (15 6 p. ct.)	Under 2 yrs. 87 22		2–4 years. 146 25		4–6 years. 116 20		6–8 years. 79 9		8–10 years. 58 3		20 2	15 2	..	Of 4 cured cases the ages are not stated.
v. Widerhofer	100 24 (24 p. ct.)	8 5	24 9	20 7	14 0	16 3	5 0	7 0	2 0	1 0	..	2 0	1 0	..	Deutsche med. Wochen., Jan. 10, 1895.
Schröder	63 8 (12.7 p. ct.)	Under 2 yrs. 6 3		2–10 years. 52 4								Over 10 years. 5 1			
Bokai	120 31 (25.5 p. ct.)	1 1	22 10	22 7	24 4	18 3	13 1	6 1	7 3	2 0	1 0	4 1	..	..	69 cases were under 3 years of age.
Heim	27 6 (22 p. ct.)	..	5 1	4 1	2 0	6 2	3 0	2 0	2 2	..	..	3 0			
Unterholzner	31 8 (25.8 p. ct.)	2 1	9 5	7 2	3 0	2 0	1 0	2 0	2 0	8–13 years. 3 0				..	Of 21 under 4 years of age, 13 recovered.
Börger	30 2 (6.6 p. ct.)	..	..	1 0	2 0	1 0	1 1	3 0	2 0	2 0	2 0	3 1	2 0	11 0	16 cases were 10 years old or over.
Hager	25 1 (4 p. ct.)	1 0	1 0	1 0	3 0	6 1	4 0	Over 6 years. 9 0							
Seiz	27 1 (3.6 p. ct.)	1 0	4 0	2 1	6 0	4 0	4 0	1 0	2 0	3 0	..	..	..	..	Private practice.
Seitz	35 2 (5.7 p. ct.)	Under 4 years. 18 2				4–6 years. 10 0		7–15 years. 7 0						..	One fatal case was 13 months, the other 18 months old; both with laryngeal diphtheria.
Reported in Brit. Med. Journ., Feb. 2, 1895.	95 22 (23.2 p. ct.)	4 2	9 3	6 1	12 1	10 3	5–10 years. 23 9					10–15 years. 15 2		16 1	

TABLE III—*Continued*

Reporter	Total cases	Und. 1 yr.	1–2 years	2–3 years	3–4 years	4–5 years	5–6 years	6–7 years	7–8 years	8–9 years	9–10 years	10–12 years	12–15 years	Over 15 yrs.	Remarks
Goodall........	72 14 (19.4 p. ct.)	4 1	10 2	7 1	9 3	10 5	22 2					10 0		..	Brit. Med. Journ., Jan. 12, 1895. These are the ages of cases previously reported by Washbourn, Goodall, and Card.
Hall...........	11 3 (27.3 p. ct.)	..	1 0	..	..	..	..	1 1	..	1 1	..	1 0	2 1	5 0	
Epidemic in Trieste, hosp. cases.	105 27 (25.7 p. ct.)	9 5	12 5	12 6	15 3	16 5	13 1	7 0	6 1	4 0	2 1	6 0	..	3 0	
Epidemic in Trieste, private practice.	72 5 (6.9 p. ct.)	..	2 0	3 2	8 1	11 1	12 0	5 0	1 0	7 1	2 0	3 0	7 0	1 0	Ten cured cases are not accounted for in this series.
Blumenfeld....	50 2 (4 p. ct.)	..	6 1	5 0	..	5 0	5 0	14 0	5 1		..	10–13 years. 9 0		1 0	Private practice.
Schaewen......	15 0	..	1 0	..	..	1 0	2 0	..	3 0	..	3 0	2 0	2 0	1 0	Private practice.
Rapmund......	100 7 (7 p. ct.)	1 0	6 1	12 2	3–5 years 22 1		5–10 years. 43 3					10–20 yrs. 15 0		Over 20 1 0	Private practice. 14 cases were under treatment at time of report.
v. Muralt......	58 2 (3.4 p. ct.)	..	1–3 years. 21 1		Over 3 years. 37 1										The latter fatal case was of a tracheotomized child, 4 years old, admitted on the sixth day of the disease; the former case was of an infant, 2 years old, with syphilis, measles, and pneumonia.
Codd & Whitehouse.	11 4 (36.4 p. ct.)	1 0	..	1 1	..	1 1	1 0	1 0	..	2 0	2 0	..	1 1	1 1	The fatal case was a woman, 39 years old, admitted in a state of collapse the 8th day of the disease, and dying in 24 hours.
Winkfield......	22 4 (18.2 p. ct.)	..	1 1	2 1	..	4 0	2 1	3 1	2 0	3 0	..	2 0	1 0	2 0	
Van Nes.......	52 12 (23 p. ct.)	..	1 0	7 4	4 2	9 4	8 0	7 0	6 1	8 1	2 0	5 0			
Leichtenstern & Wendelstadt.	123 25 (20.3 p. ct.)	0–2 years. 30 11		2–4 years. 32 6		Over 4 years. 61 8									Of 17 tracheotomized infants less than 2 years old, 9 died. Of 13 unoperated patients less than 2 years old, 2 died. Of 11 tracheotomized patients between 2 and 4 years, 4 died. Of 21 unoperated cases between 2 and 4 years, 2 died.

of the children, or the number of cases under a certain age, or the number between arbitrarily selected limits of age, etc., so that many of the reports were not used for the following table. In each space in the table the upper number is the total number of the cases belonging to the heading, and the lower number is the number of deaths among these cases.

As the cases in the preceding table were not classified according to the ages by a uniform plan in the different reports, they cannot all be summarized in a single table, but the chief results can be presented as follows:

14 reports	Total	0–2 years	2–4 years	4–6 years	6–8 years	8–10 years	10–12 years	12–15 years	Over 15 yrs.	Undetermined
Cases	1234	187	327	297	176	114	63	32	24	(14)
Deaths	215	60	70	48	19	8	4	5	1	(0)
Percentages	17.4	32.1	21.4	16.2	10.8	7	0.63	1.6	4.1	(0)

In the following table the cases under four years are from twenty reports containing 1630 cases (fatality 17.6 per cent, and those over four years are from seventeen reports containing 1451 cases (fatality 17.4 per cent).

	0–2 years	2–4 years	4–10 years	10–15 years	Over 15 years
Cases	263	411	681	129	40
Deaths	86	84	95	11	2
Percentages	32.7	20.4	13.9	8.5	5

The following table gives the results for each year up to five and over five years.

18 reports	Total	Under 1 year	1–2 years	2–3 years	3–4 years	4–5 years	Over 5 years	Undetermined
Cases	982	34	112	118	116	140	452	(10)
Deaths	179	16	37	36	17	31	42	(0)
Percentages	18.2	47.1	33	30.5	14.7	22.1	9.3	(0)

The table furthermore shows under one year 35 cases, with 16 deaths, or 45.7 per cent; under two years 291 cases, with 97 deaths, or 33.3 per cent; under three years 304 cases, with 93 deaths, or 30.6 per cent; and under four years 692 cases, with 122 deaths, or 17.6 per cent (each of these four groups of cases being from a total number of cases in the first group of 1082 cases, in the second group of 1914 cases, in the third group of 1140 cases, and in the fourth group of 1665 cases, the average fatality for the whole number of cases being 17.3 per cent).

The most frequently quoted percentages of fatality in diphtheria, according to the age, are those of Herz, and are as follows:

Under 1 year	80	per cent
1-3 years	45	“
3-5 “	40	“
5-10 “	17	“
Over 10 years	17	“

The percentage of fatality in V. Hirsch's statistics of 2658 cases from the surgical clinic in Berlin, for ten years and seven months (ending July 31, 1894), according to age, are:

Under 1 year	88.3	per cent
1- 2 years	82.5	“
3- 4 “	63.9	“
4- 5 “	56.6	“
5- 6 “	46.9	“
6- 7 “	43.7	“
7- 8 “	36.1	“
8- 9 “	28.1	“
9-10 “	31.1	“
10-11 “	21.2	“
11-12 “	20.9	“
12-13 “	18.5	“
13-14 “	16.7	“
14-15 “	15	“
15-16 “	12.5	“
Adults (72 cases)	11.1	“

Baginsky gives the following percentages from his service in the Kaiser- und Kaiserin-Friederich Children's Hospital, in Berlin, as the mean of the four years 1890 to 1893 inclusive:

Under 2 years	60.2	per cent
2- 4 years	51.2	“
4- 6 “	38	“
6- 8 “	28.9	“
8-10 “	24.5	“
10-12 “	28.8	“
12-14 “	18.5	“

Baginsky's results in Table III may be compared with this last list of percentages, otherwise I do not consider that these statistics of Herz, Hirsch, and Baginsky furnished any certain standard of comparison for the percentages of fatality derived from Table III. I have cited them, however, in the absence of any such standard, to show in a general way that these latter percentages indicate a low fatality according to age. The contrast between a fatality percentage of 33.3 for cases of diphtheria under two years of age treated with serum, and that of 60 to over 80 for cases of the same age not so treated is a striking one, even if a large allowance be made for differences in the characters of the cases in the two groups.[9]

We come now to the consideration of the influence upon the fatality of the length of the interval between the onset of diphtheria and the first injection of antitoxin. In experiments upon animals this factor is decisive in determining the result. It is the factor which Behring from the first has put in the foreground. His claim is that no death will occur from diphtheria if antitoxin is injected in sufficient dose at the beginning of the disease, and that the fatality will fall under 5 per cent if the treatment in proper manner is begun before the third day of the disease.

[9] A second provisional report of the collective investigation instituted by the Deutsche medicinische Wochenschrift, has been published (August 8, 1895), but too late for consideration in the text of this article. Of 5833 cases of diphtheria treated with serum, tracheotomy was performed in only 5.4 per cent of the cases. This material evidently is not well comparable with the great mass of statistics, chiefly from hospitals, already reported, but is probably representative of the experience in private practice. Of the cases treated with serum 12.6 per cent were under two years of age; 69.1 per cent were between two and ten years; and 18.3 per cent were over ten years. Of the 4479 cases treated without serum, 11.1 per cent were under two years; 60.5 per cent were between two and ten years; and 28.4 per cent were over ten years of age. The total fatality of the cases treated with serum was 9.6 per cent, that of cases treated without serum was 14.7 per cent. The significance of this difference in favor of serum is increased by the fact that 81.7 per cent of the serum treated cases were not over ten years of age, whereas only 71.6 per cent of the cases treated without serum were not over ten years.

The fatality of the serum treated cases under two years of age was 21.8 per cent (11.8 per cent when the treatment was begun on the first and second days of the disease), that of the cases treated without serum under two years was 39.7 per cent. The corresponding figures for ages between two and ten are for serum 8.8 per cent, and without serum 15.2 per cent; over ten years of age, for serum, 4.1 per cent, without serum 3.7 per cent. These results would indicate that the benefits of the serum treatment are greatest in infants and are not apparent in adults. I think it is probable that the benefits of the treatment are most strikingly manifested in infants and young children, but other reports show that adults are also benefited.

A fuller report of these cases is promised.

Of course, the only significance of this great emphasis upon the importance of early treatment is an expression of the fact that cure is rendered more difficult the larger the number of the diphtheria bacilli, the greater the amount and intensity of their toxins, the greater the damage already inflicted by the bacilli and their toxins, and the more serious the complications and secondary infections. There is, however, no absolute parity between the length of time the disease has lasted before beginning treatment and the increase of these dangers. One case may become desperate within forty-eight hours after the onset, and another may present no grave symptoms after a week's duration. The virulence, the number, and the microbic associations of the infecting bacilli, and especially the local and general susceptibilities of the patient, are factors no less important than the single factor of time in influencing the issue.

The individual peculiarities of each case must be considered. If all is judged according to one simple uniform standard—antitoxin cures the case or it does not cure the case—and it must be confessed this is all which seems to be in the minds of many, then the practitioner will not come to any clear conception of the wonderful powers of the healing serum. The sins of some observers in this matter seem incredible. They lump together indiscriminately all of their cases, including those complicated with measles, scarlet fever, tuberculosis, and other diseases, the mixed infections, the anginas, the croups, the advanced and the early cases, the true and the false diphtherias, the infants and the adults, and throw them into the scale to be weighed for or against antitoxin. An unsuccessful case is put down to the discredit of antitoxin without reference to its peculiarities. On the basis of experience in treating a dozen cases the writer bodily attacks results established by the careful observations of hundreds of cases. It is true we need these brute figures for comparison with former fatality statistics of diphtheria, and they have served to demonstrate the curative efficacy of antitoxin; but reports of personal experience with the serum treatment should at least contain the data for an intelligent analysis of the cases treated. Such analysis is requisite in order to reveal the full scope and capabilities of the new treatment. We have already seen that the study of the cases with reference to laryngeal involvement has brought to light evidence in favor of the serum treatment more convincing than that derived from the gross statistics of all cases treated, and evidence of a kind which meets many of the objections which have been urged against the interpretation of the gross statistics as demonstrative of the efficacy of antitoxin. We shall now see that the analysis of the cases according to the day of the disease on which the serum treatment is begun almost, if not completely, substantiates Behring's original claims, astounding as they seemed to be.

TABLE IV

Fatality according to the Day of Disease upon which Antitoxin is Injected

In each space the higher number is the total number of cases treated on the corresponding day, and the lower number is that of the deaths

Reporter	Total number of cases	1st day	2d day	3d day	4th day	5th day	6th day	After 6th day	Undetermined	Remarks
Kossel	117 13 (11.1 p. ct.)	14 0	30 1	29 0	9 1	11 2	6 3	12 5	6 1	Fatality for first three days was 1.4 per cent.
Körte	121 40 (33.1 p. ct.)	..	..	..	..	..	..	..	..	Of 37 severe and moderately severe cases injected during the first three days of the disease, 8 died (21.6 per cent). The results following injection begun after the third day were less favorable.
Baginsky	525 83 (15.6 p. ct.)	111 3	134 14	92 13	52 12	39 14	13 4	29 12	55 11	All of the three fatal cases of the first day were far advanced on admission, therefore the statements of the parents as to the date of beginning of the disease were probably erroneous (Baginsky). Mortality for first three days was 8.9 per cent.
v. Mering	74 4 (5 p. ct.)	..	..	..	..	..	..	..	..	Treatment begun on the 1st or 2d day in nearly all cases.
v. Noorden	81 19 (23 p. ct.)	..	..	..	..	..	..	..	..	Treatment begun on the 3d or later day in nearly all cases.
Schröder	63 8 (12.7 p. ct.)	23 1		27 3		13 4				
Vierordt	55 8 (14.6 p. ct.)	3 0	14 2	17 2	9 0	7 3	1 0	4 1		
Rumpf	26 2 (8 p. ct.)	..	18 1	3 1	5 0		..	..	..	Four cases were still under treatment at date of report.
Ganghofner	110 14 (12.7 p. ct.)	3 0	30 2	35 3	18 4	9 3	2 0	13 2		
Heim	27 6 (22 p. ct.)	..	9 0	2 1	7 1	3 1	1 0	5 3		
Börger	30 2 (6.6 p. ct.)	3 0	13 0	9 0	..	3 1	1 0	1 1		
Hager	25 1 (4 p. ct.)	14 1	5 0	4 0	1 0	..	..	1 0	..	Private practice. The single fatal case died of complications after cessation of the diphtheria.
Kuntzen	25 3 (12 p. ct.)	3 0	6 0	7 1	2 0	2 0	1 1	4 1		
Schmidt	14 3 (21.4 p. ct.)	..	..	..	..	..	..	..	..	The three fatal cases were not treated until after the disease had existed for 8 to 14 days. Private practice.
Seitz	35 2 (5.7 p. ct.)	..	10	12	9	4		..		The two deaths were infants 1½ years and 13 months old, with advanced laryngeal diphtheria on day of admission, presumably, therefore, treated after 2d or 3d day, although this is not stated.
Hall	11 3 (27.3 p. ct.)	2 0	4 0	4 2	..	1 1	..	..	..	A girl, 10 years old, whose brother and sister had recently died of diphtheria, developed diphtheria while under observation in the hospital. Antitoxin was at once injected, and the next day membrane was smaller, temp. normal, and recovery promptly followed.
Epidemic in Trieste, hospital cases.	105 27 (25.7 p. ct.)	6 0	30 5	29 9	20 5	11 4	7 3	2 1		
Epedemic in Trieste, private practice.	72 5 (6.9 p. ct.)	14 0	27 2	18 1	8 2	2 0	2 0	1 0		

TABLE IV—*Continued*

Reporter	Total number of cases	1st day	2d day	3d day	4th day	5th day	6th day	After 6th day	Undetermined	Remarks
Witthauer.....	36	4	8	4	6	3	..	11		
	5 (14 p. ct.)	0	2	1	0	0		2		
Blumenfeld....	50	40		8		2	..	..	..	Of the two fatal cases, in one the treatment began on the 4th and in the other on the 5th day. Private practice.
	2 (4 p. ct.)	0		1		1				
Rapmund......	100	39	34	15	5	3	1	3	..	The deaths are given for the 1st and 2d, the 3d and 4th and the 5th, 6th and after 6th day respectively.
	7 (7 p. ct.)	2		3		2				
Schaewen......	15	6	3	..	..	1	..	..	5	Of the 5 undetermined cases the statement is that they had been ill several days before the injection.
	0	0	0			0			0	
Risel	114	..	78	21	4	4	5	..	2	Eighty-nine of these cases were treated at their homes, and 25 in hospitals. They were mostly children.
	9 (7.9 p. ct.)		4	4	0	0	1		0	
v. Muralt......	58	11	18	17	After 3d day 12					
	2 (3.4 p. ct.)	0	0	0	2					
Codd and Whitehouse.	11	1	2	5	..	2	..	1		
	4 (36.4 p. ct.)	0	0	2		1		1		
Winkfield......	22	..	8	7	4	2	..	1	..	Of the 2 fatal cases in which it is stated the injection was made on the second day, in one there were on admission advanced laryngeal and nasal diphtheria with glandular swellings, and in the other thick membranes in the throat, nasal discharge, and glandular swellings.
	4 (18.2 p. ct.)		2	1	1	0		0		
Howard........	40	24	7	2	2	3	2	..	..	Private practice.
	3 (7.5 p. ct.)	0	0	0	1	0	2			
Van Nes	52	2	13	10	7	8	..	12	..	No details given of fatal case said to be treated on first day of the disease, but it is noted that the determination of the day was very uncertain in the class of patients (children of laborers) admitted.
	12 (23 p. ct.)	1	1	1	2	3		4		
Kurth	97	12	35	16	19	5	3	7	..	Of the 2 fatal cases in which it is stated the injection began on the 2d day, one was admitted with croup, and died 18 hours afterward; the other died a month after disappearance of the local diphtheria with albuminuria and bronchopneumonia.
	10 (10.3 p. ct.)	0	2	1	3	1	1	2		

There is, of course, in many cases considerable uncertainty as to the exact duration of the disease at the time when the patient is first seen by the physician. The statements of parents, or of those in charge of the children, are often the only evidence on this point which can be obtained. Satisfactory information will be particularly difficult to obtain in the class of patients in the diphtheria wards of hospitals, these patients being chiefly the children of laborers. We are also to consider that a diphtheric affection of the throat may exist without such manifest disturbance as to attract even intelligent observation, or it may be mistaken for a simple sore throat. A tabulation of cases of diphtheria according to the day of beginning treatment will be, therefore, only of relative value; but we may fairly assume that the duration of the disease will very rarely, if ever, be shorter, but often longer than that stated.

In the excellent reports on antitoxin treatment from the Paris hospitals the cases are not analyzed according to the day of beginning treatment, as Roux, whose scheme of classification has been followed by most other French writers, stated in his original article that it was practically impossible to obtain trustworthy statements on this point from parents of the children. Most of the reports, therefore, which enter into Table IV, are from German and English sources.

The statements as to the day of the disease are entirely from information obtained from parents and others, and are not estimates on the part of the physician, although in several instances the reporter says that the condition of the patient plainly indicated a longer duration of the disease than that assigned by the parents and put down in the report. It will be observed that not all of the reports in the table fit into any one system of classification, and therefore, not all can be summarized in a single table. In each space the higher number is the total number of cases belonging to the heading, and the lower is the corresponding number of deaths.

The following table is the summary of the 19 reports of the preceding table, in which the number of the cases, with the results corresponding to the day of the disease on which antitoxin treatment was begun, is given for each day up to and after the sixth day:

19 reports	Total	1st day	2d day	3d day	4th day	5th day	6th day	After 6th day	Undetermined
Cases	1489	222	456	311	168	116	44	104	(68)
Deaths	212	5	37	42	32	34	15	35	(12)
Percentages	14.2	2.2	8.1	13.5	19	29.3	34.1	33.7	(17.6)

Including with the preceding 19 reports those of Schröder, Blumenfeld, and Rapmund, we have the following table, which gives the results of antitoxin treatment begun on the first and second, on the third and fourth, and after the fourth day.

22 reports	Total	1st and 2d day	3d and 4th day	After 4th day	Undetermined
Cases	1702	814	534	286	(68)
Deaths	229	45	81	91	(12)
Percentages	13.5	5.5	15.2	31.8	(17.6)

It may also be computed from the table that of 1729 cases of diphtheria with a fatality of 14.9 per cent, 1115 cases treated with antitoxin during the first three days of the disease yielded a fatality of 8.5 per cent, whereas 546 cases in which antitoxin was first injected after the third day of the disease yielded a fatality of 27.8 per cent. Of 232 cases in which treatment was begun on the first day, 5 (2.15 per cent) died; of 492 cases in which treatment was begun on the second day, 38 (7.7 per cent) died; of 331 cases in which treatment was begun on the third day, 43 (13 per cent) died.

As is well known, the fatality from diphtheria by any approved method of treatment is smaller the earlier in the disease the treatment is begun. This is clearly shown in the following table from the statistics of V. Hirsch of the cases treated in the surgical clinic in Berlin for ten years preceding August, 1894, and, of course, before the employment of antitoxin. The results are according to the day of the disease on which treatment was begun.

	1st day	2d day	3d day	4th day	5th day	6th day	7th and 8th day	After 8th day
Cases................	241	405	323	416	203	525	506	239
Deaths..............	44	92	124	223	136	219	367	191
Percentages.........	18.3	22.7	38.1	53.6	67	67.4	72.5	81.6

The preceding table is not intended to serve as a standard of comparison for my tables giving the results of cases treated by antitoxin, as the classes of cases in the two groups are not comparable.

Philip[10] has reported from Baginsky's service the results of treatment before the use of antitoxin, begun in the earlier stages of diphtheria, the patients being brothers and sisters of children with diphtheria, who were examined for Loeffler bacilli, so that opportunity was given for recognition of the disease at its onset. The fatality was 10.5 per cent lower in those cases recognized and treated early than in the others. The fatality of the cases treated by Baginsky with serum during the first three days of the disease was 32.2 per cent lower than the preceding average fatality of cases not treated with serum. Plainly some more potent healing factor than merely that of early treatment was present. The only difference in the methods of treatment of the two groups of cases was the use of antitoxin in the one and its absence in the other.

Kohts, an opponent of the serum treatment, at the recent Congress for Internal Medicine in Munich, claims for his method of local treatment no deaths among cases treated on the first day of the disease. For later days his results are much higher than those in the serum statistics. The percentages of deaths, according to the day of beginning his treatment, as given by Kohts, without a statement of the number of cases treated, are as follows: first day, 0; second day, 20 per cent; third day, 47 per cent; fourth day, 55 per cent.

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[10] Philip: Arch. f. Kinderheilk., Bd. XVI.

Table IV shows that out of 232 cases in which it is alleged that antitoxin was injected on the first day of the disease, 5 died. As a matter of fact, however, the assumed duration of the disease in each of these fatal cases is doubtful, as it rests solely on the statements of parents or those who care for the children, and is apparently contradicted in at least the three cases, concerning which any details are given, by the condition of the patient on admission. Baginsky's three cases (Nos. 311, 479, and 511 of his table) when admitted were far advanced in the disease, with extensive membranous exudate, cyanosis, and very bad general condition. Hager's case may more readily be accepted, as it occurred in private practice; but here the patient died after disappearance of the diphtheria, from complications, whether or not referable to the diphtheria is not stated. Of the fifth fatal case reported by Van Nes no details are given in such form that the case can be identified from his description; but he himself places little reliance upon the alleged duration of the disease in the class of patients admitted to the hospital, these being the children of laborers.

I am not aware of the report of any fatal case of diphtheria properly treated by antitoxin within the first twenty-four hours after the beginning of the disease in which the duration was positively determined. A case reported by Ritter may be such, but the observation of Kurth, already cited, illustrates the difficulty of precise determination of the date of onset of diphtheria. There are, however, many such cases of prompt recovery reported as that quoted from Hall in Table IV.

It is noteworthy that the percentage of deaths in 814 cases, in which treatment was begun before the third day of the disease, is only 5.5. If we make allowance for the fact that the assigned duration of the disease can scarcely be shorter, but may readily be longer than the actual duration, then our tabulation of 1702 cases of diphtheria, according to the day of beginning treatment, nearly verifies Behring's original prediction. I do not, however, consider that it is justifiable from so small a number of cases and from material of the kind composing our table, to draw any definite conclusions as to the exact percentages of deaths according to the date of beginning treatment.

According to the table, the percentage of deaths in cases in which the serum treatment is begun on the third and fourth days of the disease is nearly three times greater than that in cases treated on the first and second days, and the percentage after the third day is three and one-fourth times greater than that of cases treated within the first three days.

We are, of course, not to infer from these results that antitoxin may not be beneficial when administered after the third and fourth days of the disease. There are cases which are still mild after this duration, but which subsequently become serious, and even in desperate cases antitoxin holds out some hope of cure.

It is apparent that the largest proportion of cures by antitoxin are to be expected from private practice among those who call the physician at an early stage of the disease. While a similar statement may be made concerning any other suitable method of treatment, it is not, I believe, true in the same measure as for the serum treatment.

The main purpose of this article has been the study of the evidence thus far published concerning the curative power of antitoxin in diphtheria. I do not propose to consider the practical points relating to the employment of antitoxin, nor to consider in detail the specific effects of injection of the healing serum. There has been much diversity of opinion as to these effects, and I shall present briefly the principal points which seem to me to be established.

Most writers approve of the continuance of such measures of local and general treatment as have hitherto been found to be useful, but recommend the avoidance of all irritating and caustic local applications.

The injection of the serum may be followed in a few hours by local pain, swelling, and redness, but there is no danger of abscess-formation if the serum is uncontaminated and proper antiseptic precautions are taken. In over 3000 injections Martin observed the formation of an abscess only three times.

In twenty-four to forty-eight hours after the injection the general condition is remarkably improved in the great majority of those patients who are in a condition to be benefited at all by antitoxin. This general improvement is accompanied by a fall of temperature, which may be a critical fall, especially if the disease is not far advanced; often it is a fall by lysis. Some hold that there may be a temporary rise of temperature as an immediate effect of the injection. Accompanying the fall of temperature is improvement of the pulse as to frequency and tension, but the heart's action may for some time, even into the period of convalescence, remain weak.

In the favorable cases the local diphtheritic process is arrested, usually within the first twenty-four hours after the injection. Membrane may appear upon spots previously inflamed and invaded by the bacilli, but otherwise there is no extension of the membrane in the majority of the cases which are benefited. The area covered by membrane becomes sharply demarcated, and the swelling of adjacent mucous membrane disappears. The membrane may disappear by rapid separation or by gradual softening. Sometimes it persists for several days after disappearance of all other local disturbance. Large membranous casts are coughed up from the larynx, trachea, and bronchi under the serum treatment more frequently than under former methods. The rapid separation of the membrane in the lower air passages may cause sudden increase of stenotic symptoms. Nasal discharge is lessened.

The swelling of the glands in the neck and the surrounding oedema disappear, so far as these are not referable to secondary infections.

The most uncertainty prevails as to the influence of antitoxin in preventing the three most important complications or sequelae of diphtheria: nephritis, heart failure, and paralysis. The weight of evidence is that genuine nephritis is far less common in cases treated by antitoxin sufficiently early than under other methods of treatment; but it is questionable whether albuminuria is less common, although it is considered to be by Kossel, Roux, and others. If there is an albuminuria in any way directly referable to the injection of the serum, and this is by no means established, it is simple albuminuria, with, perhaps, a few narrow hyaline casts, but without evidence of any serious damage to the kidney. Peptonuria, it is claimed by Hecker, is an effect of the serum, but it is without clinical significance. Albuminuria is such an extremely common symptom of diphtheria that it must be very difficult to determine that it can be referred to the serum in any case.

Many writers emphasize especially the favorable influence of antitoxin upon the heart, but there are some who have observed that with decided improvement in all other symptoms the force of the heart may still remain weak and occasion anxiety. Baginsky's experience is that the minor disturbances of the cardiac action are not less frequent in cases treated with serum—they appear to be even more frequent, as a large number of cases survive—but that actual death from heart-failure is far less common in the serum cases than in others.

Post diphtheric paralyses may occur in cases treated with serum as early as the second or third day of the disease. Whether they occur in cases treated within the first twenty-four hours is not certain. Probably they do not. According to some, paralysis is even more common in the serum cases than under former methods of treatment. This is doubtful; but if true, it may be attributed to the survival of a larger proportion of cases.

It is apparent from what has been said that antitoxin is most strikingly beneficial in progressive fibrinous diphtheria, and especially in the prevention and cure of laryngeal diphtheria. In septic diphtheria the serum treatment is of little avail.

Antitoxic serum may produce unpleasant effects, but these do not involve danger to the patient. They are in all probability referable to the serum as such and not to the healing, so-called antitoxic, substance contained in the serum. The most common undesired effect is some form of exanthem, usually erythema and urticaria, sometimes an eruption like measles or scarlatinal rash. The same exanthems have been observed by Bertin after the injection of ordinary serum of the horse, and by Richardière after injection of Marmorek's antistreptococcus serum. The serum from some horses is more likely to cause these exanthems than that from others, and there may be individual idiosyncrasies favoring their occurrence. Some writers report the occurrence of an exanthem in not more than 5 per cent of their cases, others have observed them in over 50 per cent of the cases treated with serum. They may be localized in the neighborhood of the seat of injection or extend from that over the greater part of the body, or make their first appearance at a distance from the point of injection. Often, without noticeable fever, they may be accompanied by considerable elevation and temperature and by pain and swelling in the joints. A rarer but more severe form of serum exanthem resembles erythema multiforme, and when this is accompanied, as it may be, by high fever and severe pain in the bones and joints, with swelling of the joints, the condition of the patient may really seem serious. But these patients recover. Some have attributed a petechial eruption to injection of the serum, but this may occur in diphtheria without serum treatment.

These occasional untoward effects of the healing serum are annoying, but, being unattended with danger to life and without serious consequences, they do not contra-indicate the use of the serum.

There have been a few cases reported in which the writers, without any satisfactory evidence whatever, have referred the death of the patient to the use of the serum. The essential harmlessness of the serum has been demonstrated by over a hundred thousand injections,[11] and if future investigations should show that through some idiosyncrasy on the part of the patient death ever is attributable to the injection of the serum, this would probably count for about as much as the rare deaths from the use of ether or chloroform. I shall leave untouched the question of the immunizing properties of antitoxin.

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[11] This would seem to be at least a moderate estimate, as, writing November 20, 1894, Behring says that there had been up to that date certainly over 40,000 injections (Das neue Diphtheriemittel, von Dr. Behring, Berlin, 1894, p. 25).

The principal conclusion which I would draw from this paper is that our study of the results of the treatment of over seven thousand cases of diphtheria by antitoxin demonstrate beyond all reasonable doubt that antidiphtheric serum is a specific curative agent for diphtheria, surpassing in its efficacy all other known methods of treatment for this disease. It is the duty of the physician to use it.

The later reports show in general a decided improvement in the results of the treatment over the earlier ones, and there is every reason to believe that the results of the second year's employment of the new treatment will make a much more favorable showing than those of the first year. We shall come to a clearer understanding of the mode of action of the healing serum. Improvements in the methods of preparation and preservation of the serum, and possibly the separation of the healing substance, at least from other ingredients which produce the undesired effects, may be expected.

The discovery of the healing serum is entirely the result of laboratory work. It is an outcome of the studies of immunity. In no sense was the discovery an accidental one. Every step leading to it can be traced, and every step was taken with a definite purpose and to solve a definite problem.

These studies and the resulting discoveries mark an epoch in the history of medicine. It should be forcibly brought home to those whose philozoic sentiments outweigh sentiments of true philanthropy that these discoveries, which have led to the saving of untold thousands of human lives, have been gained by the sacrifice of the lives of thousands of animals, and by no possibility could have been made without experimentation upon animals.

CLINICAL AND BACTERIOLOGICAL DIAGNOSIS OF DIPHTHERIA[1]

As I was unable to be present at the morning session, I will take advantage of this opportunity to express my conviction of the great significance of this Conference. It inaugurates an important movement in the interests of public health in this state. By bringing together persons from various professions and walks of life, it will spread an intelligent interest in sanitary matters; it will lead to the education of the general public as to the importance of public hygiene and it should secure cooperation on the part of the great body of physicians with the efforts of municipal and state boards of health. There should result an educated public sentiment to support well directed efforts of the officers of public health, to demand new sanitary legislation when needed and adequate means to carry out sanitary regulations and to aid in the solution of sanitary problems. The success of this first conference will, I trust, lead to a permanent organization with similar purposes and widened scope, an organization capable of being of material assistance to the various boards of health throughout this state.

To turn to the theme which has been assigned to me on this occasion, I must express a certain feeling of hesitation in talking before a mixed audience about the harmful bacteria. A half knowledge concerning the living germs of disease is calculated to give rise to exaggerated and alarming apprehensions which a fuller knowledge of the subject would correct. The general public entertains sentiments of great animosity toward all those members of the vegetable kingdom which are called " bacteria." They hear only of those bacteria which cause disease and little or nothing of the vastly greater number of bacterial species which are not at all harmful and many of which, indeed, are of the utmost service to mankind. The very existence and continuance of life upon this globe are dependent upon the activities of these lowly and much abused organisms. One might, with equal justice, cherish hostile feelings towards all of the higher plants because among them are a few poisonous species. The friendly bacteria have not received their deserts in public estimation. The mere statement that water, or milk, or various substances with which we come into contact, contain so many bacteria is of very little significance. It all depends upon whether harmful bacteria are present and, fortunately, such bacteria are the exception and not the rule. Again, in this preface to what I have to say, I would call to your minds that we are fortunately provided by nature with admirable and manifold means of defence against the invasion and injurious action of even most of the harmful bacteria which we may chance to receive.

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[1] Report of remarks made at the Conference of Health Officers, Baltimore, February 17, 1897.

Maryland M. J., Balt., 1896-7, XXXVI, 392-395.

Dr. Stokes has already described to you the general characters of the diphtheria bacillus. He has demonstrated how it can be recognized and what use is made of it in the diagnosis of diphtheria, as well as many of the practical applications of this discovery. There is no longer any doubt in the minds of those who are fully informed upon the subject that the Klebs-Löffler or diphtheria bacillus is the sole specific cause of diphtheria. Every inflammation of a mucous membrane or other exposed surface that is caused by this bacillus is diphtheria, and any inflammation that is not caused by this bacillus is not diphtheria. But the mere demonstration that this organism is the cause of diphtheria simply confirms the faith of enlightened physicians that all infectious diseases are caused by microorganisms. The practitioner of medicine has the right to inquire what practical results have come from the discovery and to this inquiry it may be replied that there is perhaps no single bacterial discovery which has led to such important practical results as has that of the diphtheria bacillus. This discovery has shed light upon the causation and mode of spread of diphtheria; it has elucidated the real nature of the disease; it has furnished a positive means of recognizing the disease and distinguishing it from other affections; and, above all, it has led to a method of treatment far surpassing in efficacy all other known methods. Those who demand immediate practical results from scientific discoveries ought surely to be satisfied with the outcome in this respect from the discovery of the bacillus of diphtheria.

The old discussion as to whether diphtheria is a local or general disease has lost all significance in the light of the discovery of the bacillus of diphtheria and the study of its properties. One of the most important attributes of this bacillus is its power to produce a chemical poison of appalling potency. This poison may be compared in a general way and as to some of its properties to the poison secreted by a venomous serpent. In diphtheria the bacillus itself grows only or chiefly at the point of invasion, which is usually the throat, and in its neighborhood, where it leads to inflammation, generally with the formation of a false membrane. Here, growing only superficially in the membrane, the bacilli secrete their terrible poison or toxine, which is absorbed into the circulation and causes the grave constitutional symptoms of the disease and serious damage to remote parts, such as the heart and the kidneys. The local lesion, the false membrane, is caused directly by the bacilli; the general symptoms and distant lesions are the result of the action of the specific poison.

The subject of serum therapy or the treatment of diphtheria by antitoxin does not belong to my theme and I shall only say in this connection that the efficacy of this treatment has passed beyond the experimental stage and is settled beyond doubt. Antitoxin, where generally employed, has reduced the fatality from diphtheria at least fifty per cent. Thousands of lives have already been saved by its use and countless thousands will be saved in the future by a discovery resting entirely upon the results of experimentation upon animals.

The diphtheria bacillus affords a positive and practically unfailing means of diagnosis of the disease diphtheria and it is more particularly to this aspect of the subject that I have been requested, in the division of the general theme, to direct your attention. The possibility of this accurate diagnosis signifies much for the practitioner and for the patient.

Before the discovery of the bacillus of diphtheria the disease was diagnosed by certain symptoms and lesions, the most characteristic feature being the presence of a false membrane. This constitutes the clinical diagnosis of diphtheria and it still remains the most available method of diagnosis for the great body of medical practitioners. The bacteriological diagnosis of diphtheria is not to be regarded as intended to supplant the clinical diagnosis or in any sense as antagonistic to the clinical method. It is simply a valuable additional aid in diagnosis, in many cases simply confirmatory of a diagnosis reasonably certain upon purely clinical grounds, and in doubtful cases of decisive importance. For the scientific study of many problems relating to diphtheria bacteriological diagnoses of all cases studied are essential.

The question is of much practical importance whether the diagnosis of diphtheria by bacteriological methods necesssitates any material readjustment of the views which had been reached by the anatomical and clinical study of the disease. In my opinion no such readjustment of these views is required as would appear from some of the writings upon this subject. Our experience here in Baltimore has been that over 90 per cent of the primary pseudomembranous inflammations of the throat, which the physician upon clinical grounds alone would confidently diagnose as diphtheria, are in fact genuine diphtheria capable of demonstration as such by the detection of the Klebs-Löffler bacillus. Statements, based upon the examination of large series of suspected cases of diphtheria, to the effect that not more than

60 to 75 per cent of the cases are genuine bacillar diphtheria, are in a measure misleading and it is not to be understood that all of these suspected cases relate to primary, pseudomembranous inflammations about the nature of which the practitioner would not be in doubt upon clinical grounds. It sometimes requires repeated, painstaking examination to detect the diphtheria bacilli in diphtheric exudates, although, as a rule, they can be found without much difficulty. So far, then, as these primary pseudomembranous inflammations of the throat are concerned, no important readjustment of diagnosis is required as the result of bacteriological studies. Not a few, however, of the pseudomembranous inflammations of the throat secondary to scarlet fever and other acute infections are due to other organisms than the Löffler bacillus and are, therefore, not true diphtheria. The primary membranous croups are nearly all diphtheria.

But it is in the doubtful cases, and more particularly in the milder inflammations of the throat with little or no false membrane, that the bacteriological diagnosis is of prime service. Here the clinical diagnosis alone is generally not decisive. Some have been very reluctant to include these mild cases under diphtheria, but the conception that diphtheria may manifest itself in the form of mild, nonmembranous inflammations was not introduced by the bacteriologists. There were not a few excellent clinicians who advocated this doctrine long before the bacteriological era. One sometimes hears today the statement that bacteriologists demand that every throat harboring the Löffler bacillus should be regarded as affected with diphtheria. Such a view is as ridiculous as to consider the presence of the streptococcus upon the healthy skin as indicative of erysipelas. The bacillus must not only be present but it must be doing harm by unfolding its pathogenic activities, that is by setting up inflammation. The whole point, however, is that this inflammation may be mild, without membrane, as well as severe, necrotic, with membrane, and the mild, nonmembranous inflammations are just as truly diphtheria as are the membranous types. The presence of diphtheria bacilli in healthy throats, which have not recently been the seat of diphtheria or which do not subsequently become diphtheric, is a rare occurrence. The recognition of the mild cases of diphtheria, which can be positively diagnosed only by bacteriological examination, is of no little practical importance, for such mild cases may become severe and they are capable of spreading the disease to others, even in malignant form.

The physician will do well during periods of prevalence of diphtheria to consider all sore throats in children, certainly all in households where undoubtedly diphtheria exists, as suspicious of diphtheria. The explanation of the relative mildness of the inflammation in some cases of infection with the diphtheric bacillus may sometimes be the weakened virulence of the infecting bacillus, but it is more frequently attributable to more than usual resistance on the part of the individual to this organism.

As the chairman of this meeting has alluded to the prevalent belief that cats may acquire diphtheria and be the means of transmitting it to human beings, I may be permitted to touch upon this point, although it is not strictly relevant to my theme. Noah Webster, in his curious book on "Epidemic and Pestilential Diseases," published at the end of the last century, noted the coincidence of cat distempers with malignant sore throat. More recently Klein has brought together the evidence on this point and thinks that observations which he has made support the popular belief, but his observations do not seem to me convincing. In an address before the Medical and Chirurgical Faculty of this state about five years ago, I referred to this matter and expressed a desire to make bacteriological examinations of cats suspected to have diphtheria or to be agents of conveyance of the disease, but no opportunity for such examination has presented itself. In my judgment there is no conclusive evidence that cats are ever spontaneously infected with the diphtheria bacillus, although they are susceptible to experimental inoculation with it.

It is not to be expected that the practitioner of medicine, as a rule, will himself make bacteriological examinations in cases of suspected diphtheria. Relatively few have either the training or the appliances for such examinations, even if they have the time. Students who are now educated in our best medical schools are taught bacteriological methods and in their future practices should be able to make such examinations as those required for the bacteriological diagnosis of diphtheria. From what has been said, the clinical diagnosis in many cases is sufficiently positive for all practical purposes. The physician should not delay the use of antitoxin in suspected cases of diphtheria in order to await the results of bacteriological examination. There remain, however, a sufficient number of cases where it is of the utmost importance that means should be at the disposal of the physician through which he can secure the advantages of bacteriological examinations by skilled experts. Nor is it simply for purposes of diagnosis that such examinations may be required.

I would, therefore, in conclusion, emphasize the great value to the medical profession and to the interests of public health of the establishment of well equipped and properly directed bacteriological laboratories in connection with the municipal and state boards of health.

A model in this respect is the laboratory of the Health Department of New York City, which has already accomplished results demonstrating the great benefits to the medical profession and the general public of such laboratories.

The impulse for the establishment of this laboratory was the cholera scare a few years ago. The fear of Asiatic cholera has been one of the great levers of sanitary reform in this century. Here in Baltimore a bacteriological laboratory has been started in connection with the Health Department. It should receive the hearty support of the medical profession and its capacity for usefulness should be extended by ampler provisions for its support, whereby it may be made more serviceable not only to this city but to the entire state.

BACILLUS COLI COMMUNIS[1]

THE CONDITIONS OF ITS INVASION OF THE HUMAN BODY, AND ITS PATHOGENIC PROPERTIES

We owe especially to Escherich the knowledge that certain species of bacteria are regularly found in the healthy intestinal canal. Of the bacteria present in the normal faeces the most abundant is *Bacillus coli communis*, first described by Escherich in 1885, under the name *Bacterium coli commune.*

This bacillus presents so many points of resemblance to the typhoid bacillus that Rodet and G. Roux contend that the latter is simply a variety or modified form of the colon bacillus. There are, however, so many points of difference between these two bacilli that they must be regarded as distinct species. The typhoid bacillus is actively motile, the colon bacillus only feebly motile; the typhoid bacillus never coagulates milk, the colon bacillus coagulates it in two to seven days; the typhoid bacillus does not cause fermentation of lactose, or if at all, only in very feeble degree, while the colon bacillus actively ferments lactose. There are also differences in the appearances of the growth of the two bacilli on gelatin, agar and potato, but these are less precise and constant than the points mentioned.

Escherich demonstrated that injections of bouillon cultures of the colon bacillus into the circulation of guinea pigs and rabbits are capable of killing animals by acute intoxication, if sufficient quantity is injected. Smaller quantities he believed to be devoid of pathogenic power. Dr. Blachstein has published the results of a series of experiments made in my laboratory, showing that injections of 1 c. c., or even less, of bouillon cultures of the colon bacillus into the ear-veins of rabbits may cause the death of the animals as late as six weeks after the inoculation. These rabbits were much wasted, and presented peculiar changes in the liver and bile. The bile contained the colon bacilli in large number, at a time when they had disappeared from the blood and organs. It was thin and pale, devoid of the normal green color, and presented particles of bile pigment, necrotic epithelium, and masses of bacilli. The liver often contained necrotic foci; the spleen was atrophied.

The first observation of the colon bacillus in the tissues of the human body outside of the intestinal canal was made in 1889 by Tavel, who found this

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[1] Report of President's address (abstract) before the semi-annual meeting of the Medical and Chirurgical Faculty of the State of Maryland, Rockville, Md., November 17, 1891.

Med. News, Phila., 1891, LIX, 669-671.

organism in the wound resulting from removal of a tumor of a thyroid gland. Since then there have been several observations of this bacillus associated with peritonitis, angiocholitis, and some other affections. Observations of this invasion of the colon bacillus have been made by Wyss, Laruelle, Gilbert and Girode, Charrin and Roger, Naunyn, A. Fränkel, and Malvoz. Bönnecken has found the colon bacillus in the fluid of strangulated hernial sacs.

No systematic observations, however, have been published as to the frequency and conditions of invasion of this bacillus into the tissues of the human body. I have for about a year and a half been interested in observations of this character, and take this opportunity to present the most important of the results.

My first observation was in April, 1890. The case was one of acute hemorrhagic pancreatitis, with multiple fat-necroses in the mesocolon and omentum. The bile was nearly colorless, and presented a sediment containing yellow, granular bile pigment, cylindrical epithelium, and zoöglea of bacilli, reminding one of the changes of the bile observed in rabbits dying a long time after intravenous inoculation with the colon bacilli. There existed a very acute diphtheritic colitis with superficial ulceration. In this case pure cultures of the colon bacillus were obtained from the foci of fat-necrosis, the mesenteric glands, the liver, the bile, the lungs, the spleen, and the kidneys. In this, as in all the cases, roll agar and gelatin cultures were made after thoroughly burning with a hot knife the surface of the organ at the point where the sterilized needle was inserted. The autopsy was made within one hour after death, and in general the autopsies were made in less than twelve hours after death. The colon bacillus, moreover, is not one of the bacilli that invades organs after death in the process of postmortem decomposition.

As in this case diphtheritic and ulcerative colitis existed, it seemed probable that it was the lesion of the intestinal mucosa that permitted the invasion of the colon bacillus. From this time we have made bacteriological examinations in the manner described in most of our autopsies.

We have now found the colon bacillus in one or more of the organs of the body in thirty-three autopsies out of about two hundred.

The suspicion at first entertained has been abundantly confirmed, namely that lesions of the mucous membrane of the intestine open the way for the invasion of the colon bacillus into the blood and lymphatic vessels, and thence into various organs and parts of the body. The lesion consisted in different cases of haemorrhage, ulceration, perforation, catarrhal and diphtheritic inflammation, strangulation, cancer, traumatic injury, and intestinal suture.

The bacilli were found in the blood, lungs, spleen, kidneys, peritoneum, bile-ducts, gall bladder, liver, lymphatic glands, testicle, tonsil, brain, and wounds, varying in their distribution and number in different cases. They were found with especial frequency in the lungs and kidneys, but often also in the liver, mesenteric glands, and spleen. The number of colonies in an original tube varied from one or two to a countless number. The diagnosis of colon bacillus was never made without applying all necessary tests, and especially never without demonstrating the power of coagulating milk.

It is not to be inferred that ulceration or other lesion of the intestinal mucous membrane is necessarily associated with the invasion of the colon bacilli in sufficient number to be demonstrable by ordinary culture methods, but only that such invasion is a frequent result of the lesion.

In a number of cases colon bacilli were demonstrated by culture methods in various organs of the body without any noteworthy lesions of the organs containing them or any lesion that could reasonably be referred to their presence. This was true for instance in several cases of amoebic dysentery. On microscopical examination the bacilli, often in clumps, readily staining with aniline dyes and even with haematoxylin, could be demonstrated inside of small bloodvessels without any alteration in the surrounding tissues. There is, therefore, no evidence that in these cases the bacilli do any harm, although it cannot be positively stated that their presence is innocuous. It is well known that human blood serum outside of the body exerts a powerful germicidal influence upon the colon bacillus.

This class of cases, therefore, in which this bacillus appears to be a harmless invader, should make one cautious in attributing pathogenic powers to the colon bacillus, even when it is associated with definite lesions, unless it can be shown that other causes can be excluded. In the case of fat-necrosis, for instance, I do not believe that the colon bacillus was the cause of the necrosis, although both cultures and cover-slip preparations showed its presence in large number. I have subsequently made bacteriological examination of three cases of multiple fat-necrosis without finding any micro-organisms in the necrotic foci.

I have suspected that the colon bacillus may be the cause of lobular pneumonia, as in several cases this organism has been found in large number and in pure culture in congested, oedematous, and inflamed areas in the lungs. It has also been frequently associated with fatty degeneration of the kidneys, but neither in this nor in the pulmonary affection is there any conclusive evidence that the presence of the bacilli has done the harm.

The view that the changes in the bile noted in the case of pancreatitis already cited are referable to the colon bacillus, rests upon experimental evidence. In two cases of angiocholitis and cholecystitis with gall-stones, the colon bacillus was very abundant, and in pure culture, in the bile.

Naunyn has recently called attention to the possible relation between the formation of gall stones in some cases and the growth of this bacillus in the biliary passages. Gilbert and Girode, as well as Charrin and Roger, refer cases of suppurative inflammation of the bile-ducts and gall-bladder to the penetration into these parts of the colon bacillus.

In cases of peritonitis due to perforation of the intestine the colon bacillus is usually found, but not always, in large number in the exudate, sometimes in pure culture. In three cases of peritonitis due to intestinal ulceration without perforation I found the colon bacillus in large number and in pure culture. The exudate was serofibrinous, not distinctly purulent. In these cases it seems warrantable to attribute the peritonitis to the invasion of the colon bacillus into the peritoneal cavity. In a case of ruptured tubal pregnancy the bloody fluid withdrawn by a sterilized hyperdomermatic syringe from the peritoneal cavity before laparotomy was performed, yielded a pure culture of the colon bacillus. There was found a perforative appendicitis. In the pus of circumscribed abscesses due to perforation of the vermiform appendix I have found the colon bacillus in nearly pure culture. It is a mistake, however, to say, as Malvoz has recently done, that all cases of peritonitis due to intestinal lesion are referable to the colon bacillus. Often enough in this class of cases *Staphylococcus pyogenes aureus* or *Streptococcus pyogenes* is present—it may be predominantly so—in the peritoneum. In a case of perforative appendicitis recently examined *Streptococcus pyogenes* seemed to be the only organism present.

In a case of ovarian abscess adherent to an ulcerated cancer of the rectum the colon bacillus was found in pure culture in the abscess.

To the occasional presence of the colon bacillus in laparotomy wounds for the extirpation of diseased Fallopian tubes and ovaries, I have already called attention on another occasion.

It is especially important to know that typhoid ulceration of the intestine opens the way for the invasion of the colon bacillus, which may be found in the mesenteric glands, lungs, liver, kidneys, and elsewhere mixed with the typhoid bacillus. On account of the resemblance between these two species of bacilli a mistake might easily be made in identifying the colon with the typhoid bacillus, and there is reason to believe that this mistake has been made by some writers. The preservation of all its properties in these cases shows that the colon bacillus is not changed into the typhoid bacillus when it invades the organs in typhoid fever; and this is a further argument against the unwarrantable assumption of Rodet and G. Roux already mentioned.

Only in two cases have I found the colon bacillus in organs outside of the intestine without any demonstrated lesion of the alimentary canal. Although careful search was made, it is not improbable that some lesion was present, so small or of such a character as to escape observation with the naked eye.

ADDITIONAL NOTE CONCERNING THE INTRAVENOUS INOCULATION OF *BACILLUS TYPHI ABDOMINALIS;* ITS SURVIVAL IN THE GALL-BLADDER[1]

Since the publication of Dr. Blachstein's paper in the preceding number of this Bulletin[2] a rabbit, which had been inoculated into the ear-vein with a culture of typhoid bacillus, has been used for inoculation with another organism and typhoid bacilli have been found in the bile 128 days after the first injection. The history of this case is as follows:

March 13, 1891. A rabbit weighing 1370 grms., received by injection into the ear vein 0.5 c. c. of a bouillon culture (containing 2 per cent glucose), 48 hours old, of the typhoid bacillus. The culture was pure of the typhoid bacillus obtained originally from a single colony in a gelatine roll culture from the spleen of a case of typhoid fever. The bacillus grew invisibly on potato and in litmus milk produced a lilac pink color with acid production and without coagulation of milk. It was actively motile and presented all of the characteristics of the typhoid bacillus.

The rabbit's weight was taken daily for the month following the inoculation with the following result in grammes: 1370, 1240, 1250, 1240, 1290, 1290, 1300, 1335, 1275, 1250, 1240, 1220, 1260, 1290, 1290, 1270, 1280, 1370, 1320, 1340, 1330, 1220, 1170, 1180, 1175, 1210, 1240, 1270, 1360, 1285, 1310, 1290, 1305, 1280. During this time therefore the loss of weight was inconsiderable.

The animal presented no especial symptoms and on July 18 it was used for another experiment, namely subcutaneous inoculation with a culture of the swine-plague bacillus, an organism which kills rabbits usually within 18 hours after inoculation. The rabbit died in somewhat less than 18 hours after inoculation.

"At the autopsy the blood and organs contain in large number the small, oval, polar-staining swine-plague bacteria, but the gall-bladder and the bile present the appearances with which we were already familiar as characteristic of the presence in the gall-bladder of the typhoid bacillus and which have been described in Dr. Blachstein's article. The swine-plague bacterium produces no change in the bile and does not appear in this fluid. The gall-bladder is rather small and contracted, its walls grayish white, opaque and much thickened, measuring 0.75 mm. in thickness. The contents of the gall-bladder consist of turbid gray, rather creamy, not viscid fluid. There is no trace of bile pigment in the bile and none visible under the microscope. The mucous membrane is smooth and thick.

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[1] Johns Hopkins Hosp. Bull., Balt., 1891, II, 121-122.

[2] Blachstein: Intravenous Inoculation of Rabbits with the *Bacillus coli communis* and the *Bacillus typhi abdominalis.* Johns Hopkins Hosp. Bull., Balt., 1891, II, 96-103.

"The liver is somewhat coarsely nodulated, and presents a number of small scars on the surface, from which narrow lines of fibrous tissue extend into the liver. These fibrous scars are probably due to the passage of cysticerci, of which several are present in the peritoneal cavity (*Cysticercus pisiformis*). The acini of the liver are distinct. No psorosperm nodules or necrotic foci are visible.

"The common bile duct has thickened walls and the same turbid gray contents as the gall-bladder. This fluid can be readily squeezed into the duodenum by pressure on the common bile duct.

"The contents of the small intestine are whitish, creamy, not bile-stained; those of the caecum are greenish, yellow, normal in appearance. The rectum contains normal looking faecal balls.

"The lungs are slightly oedematous, the spleen moderately swollen; these changes being referable to the swine-plague inoculation. There are no other lesions save the acute inflammatory oedema at the seat of swine-plague inoculation.

"Cover-slip preparations of the bile show a large number (averaging about 15-30 to each field; Leitz 1-12th oil immersion) of short rather plump bacilli, with rounded ends, resembling the typhoid bacilli. The bacilli occur both singly and in clumps. In addition there are present many well formed cylindrical epithelial cells, not bile-stained, with nuclei, evidently desquamated from the wall of the gall-bladder. The bacilli are not motile when examined at once in a drop of bouillon; but in the course of five hours, at a temperature of 36° C., in a drop of culture, they developed into actively motile bacilli. No other forms of bacteria were found in the bile. In cover-slips from the blood, liver and spleen, only the swine-plague bacteria were found, which are much shorter than the typhoid bacilli, with which they cannot be confounded.

"Roll gelatine cultures from the blood, liver and spleen developed only colonies of the swine-plague bacillus, whereas similar cultures from the bile (made after thoroughly burning the surface of the gall-bladder) are pure cultures of the typhoid bacillus, no other kind of colony or organism being present in these cultures. The first tube is crowded with minute colonies; the second and third contain well distributed grayish, thin colonies, with somewhat irregular margins.

"The cultures obtained from the bile were tested in every possible way to prove their identity with those of the typhoid bacillus, and in no respect do they differ from the latter. The bacillus obtained from the bile is actively motile in cultures. It grows invisibly but well upon potato; it produces acid in milk, changing the blue lilac tint of litmus milk (of amphoteric reaction) to a lilac pink without coagulating the milk. It produces no change to the naked eye in the appearance of plain milk. The bacillus is beset on all sides with flagella when stained according to Löffler's method for staining flagella."

Typhoid bacilli found in the bile at variable periods after intravenous inoculations are always devoid of motility when examined at once, but they develop into motile bacilli.

In this case, therefore, typhoid bacilli were found in the bile of a rabbit one hundred and twenty-eight days (over four months) after intravenous injection of 0.5 c. c. bouillon culture of the same organism. During this time they had produced little or no appreciable disturbance of the animal, so far as could be judged from its general appearance during life. Whether or not the animal would have eventually wasted away and died as other animals similarly affected have done cannot be said. It presented the same changes in the gall-bladder and in the bile noticed in the other rabbits which died a long time after a similar inoculation, so that as soon as the gall-bladder was exposed at the autopsy I felt confident of finding typhoid bacilli in it.

That typhoid bacilli may make their appearance in the bile within at least 5 days after intravenous inoculation is proven by experiment recently made by Dr. Walter Reed in the Pathological Laboratory. The rabbit received 0.3 c. c. of a bouillon culture of the typhoid bacillus in the mesenteric vein, and was killed at the end of five days. The bile at the autopsy was found thinner and paler than normal, but it still had a green tint. Typhoid bacilli were found both by cover-slip preparations and by cultures in the bile in considerable number.

EXPERIMENTAL PRODUCTION OF TYPHOID-BACILLUS CARRIERS [1]

BACTERIA IN GALL-STONES

I am pleased to note that Dr. Gay has been able to confirm the observations which I made and published in part in 1891 on the experimental production of typhoid bacillus carriers. The experiments made by Dr. Blachstein were for the purpose of determining the effects of intravenous injection of colon bacilli and typhoid bacilli in rabbits, and after the preparation of his paper he left the university. The most interesting results, however, were obtained after his departure. Fortunately Dr. Blachstein had marked the rabbits which survived the intravenous injections, so that they could be identified. After about four or five months, some of the rabbits which Dr. Blachstein had marked, began to die, and I made autopsies. At first I supposed the deaths were purely accidental and that they had nothing to do with the previous inoculations. I found however, what is described in the papers to which Dr. Gay has referred; namely, that the organisms had disappeared from the blood and organs, but that there was a very remarkable condition of the gall-bladder. It was much distended with pale bile and a grumous material, which might seem to be the beginning of a rather friable gall-stone. It can be seen, therefore, that in the cases of intravenous inoculation of small doses of typhoid bacilli or colon bacilli, the dose was short of fatal. I conceived that the organism reached the gall-bladder through the necrotic liver, which occur in rabbits after intravenous injection with colon or typhoid bacilli. There, more or less withdrawn from active bactericidal substances, they simply survive indefinitely, but not without injury to the animal, which becomes emaciated and often dies after several months.

The formation of these grumous masses in the bile led me immediately to the examination of gall-stones with reference to the presence of bacteria, and I found it was not a very rare occurrence to have bacteria in the interior of gall-stones. This led me to surmise that these gall-stones were the not infrequent result of a cholecystitis and bacterial invasion.

These rabbit carriers, which we were the first to produce, as used in the experiments which Dr. Gay has made, have interested me immensely.

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[1] Remarks on a paper of Frederick P. Gay before The Johns Hopkins Hospital Medical Society Baltimore, November 2, 1914.

Johns Hopkins Hosp. Bull., Balt., 1915, XXVI, 32.

PRINCIPLES UNDERLYING THE SERUM DIAGNOSIS OF TYPHOID FEVER AND THE METHODS OF ITS APPLICATION[1]

I comply the more readily with the suggestion of the Chairman of this Section that my remarks in opening this discussion on the "Serum Diagnosis of Typhoid Fever," shall relate to the general principles of the method, inasmuch as the results obtained by this method at The Johns Hopkins Hospital will be presented in the course of this discussion by Dr. Block, and others will relate the results of their personal experience.

Before the introduction of the Widal method of diagnosis the discovery and subsequent studies of the typhoid bacillus had been comparatively barren of results available to the general practitioner of medicine. In this respect the typhoid bacillus afforded a marked contrast to many other pathogenic microorganisms, notably the tubercle bacillus, the diphtheria bacillus and the malarial parasite.

For various reasons improved methods of diagnosis of typhoid fever are most welcome to the practitioner. Inasmuch as the prevalence of this disease is a just reproach to the sanitary conditions of a locality, there has often been a readily explicable, if not creditable, reluctance on the part of some physicians, especially in public institutions, country towns and summer resorts, to make the diagnosis of typhoid fever even in clear cases. Moreover, notwithstanding all that has been written on the subject, knowledge of the frequent deviations of typhoid fever from the classical type, amounting sometimes to entire absence of all features of this type, can hardly be said to have become sufficiently common property of the medical profession of this country. The differential diagnosis of typhoid fever from certain other diseases, such as acute miliary tuberculosis, tuberculous peritonitis and meningitis, acute ulcerative endocarditis and various other septic affections, may, for a time at least, be most difficult or impossible, even to skilled diagnosticians. In children the disease is prone to anomalous manifestations. Since the discovery of the malarial parasite there is no longer any excuse for confounding typhoid and malarial fevers. A method of positive diagnosis

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[1] An address in opening the Discussion on Serum Diagnosis in the Section on Practice of Medicine, American Medical Association, Philadelphia, Pa., June 1-4, 1897.

J. Am. M. Ass., Chicago, 1897, XXIX, 301-309.

of typhoid fever will elucidate the much disputed nature of many short and mild febrile diseases and of certain fevers of warm climates, as in the southern part of this country.

Before the introduction of serum diagnosis, numerous attempts had been made to utilize the presence of the typhoid bacillus for purpose of diagnosis. One can obtain with considerable regularity cultures of the typhoid bacillus by hypodermic puncture of the spleen in typhoid patients; but this procedure is not wholly without danger. Anyone who has seen at autopsy a swollen, soft typhoid spleen with its capsule distended to the utmost and ready to burst the moment it is lifted from the body, would certainly hesitate to insert even a hypodermic needle into such a spleen during life. Instances are on record in which such puncture of the spleen has given rise to severe intraperitoneal haemorrhage. Cultures from roseola spots, suggested by Neuhauss in 1886, yield uncertain results. Cultures from the blood give positive results in some cases, especially if large amounts be used. Cultures from typhoid stools reveal the presence of the specific bacillus in many cases if the examination be made with sufficient patience and care. The introduction of the Elsner and of the Capaldi nutritive media marks a distinct advance in this method. None of these procedures, however, in their present form afford simple and ready methods of diagnosis in the routine of hospital and private practice.

History.—The Widal method of diagnosis is based on the application of scientific discoveries before Widal's first publication, June 26, 1896. As a controversy for priority, attended with no little bitterness, has arisen, it may be well to state the main historic facts. Like so many other bacteriological discoveries of practical utility, this one is the outcome of investigations concerning immunity.

In 1889, Charrin and Roger noticed that *Bacillus pyocyaneus* grows in the form of clumps in the undiluted serum of animals rendered immune from this bacillus, whereas in normal serum it grows with diffuse clouding of the medium. This is the first observation of the property of immune serum to cause agglomeration of specific bacteria.

In 1891, Metchnikoff observed the same phenomenon together with immobilization of the bacteria in cultures of *Vibrio metchnikovi,* and also clumping of the pneumococcus, in their immune sera and he said: "This fact, presenting general importance, should be investigated more fully." He did not, however, pursue the investigation, and as he failed to find the same behavior of the hog-cholera bacillus[2] in its immune serum, he seems to have abandoned the idea first expressed as to the general importance of the phenomenon.

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[2] As a matter of fact, the hog-cholera bacillus is agglutinated and immobilized by immune hog-cholera serum, as has been shown by Dawson (New York Med. Journal, Feb. 20, 1897). This is an additional proof, if any were needed, of the fact repeatedly insisted upon by the writer and others in this country, that Metchnikoff's so-called hog-cholera bacillus is not the genuine hog-cholera bacillus discovered and described by Theobald Smith (Welch and Clement, Proceedings of the 30th Annual Convention of the U. S. Veter. Med. Assoc. and 1st Veterinary Congress of America, October, 1893).

In 1893, Issaeff, in the Pasteur Institute, and in 1895, Washbourn, confirmed Metchnikoff's observation as to the pneumococcus, and in 1894 Issaeff and Ivanoff, in Koch's Institute, made the same observation regarding the vibrio of Ivanoff.

In 1894, Pfeiffer, in conjunction with Issaeff, published his important studies on immunity from Asiatic cholera, in which he showed that cholera spirilla introduced into the peritoneal cavity of immunized guinea pigs, or introduced together with immune serum into the peritoneal cavity of normal guinea pigs, quickly lose their motility and break up into small granules. This behavior in the animal body, known as the "Pfeiffer phenomenon," and demonstrated in 1896 by Pfeiffer and Kolle also for typhoid infection, became the subject of investigation by others, which led to the recognition of the importance and general bearings of the agglutinative reaction of specific sera. The Pfeiffer phenomenon is distinct from the agglutinative reaction upon which the method of serum diagnosis to be here considered is based, and Pfeiffer himself cannot be credited with a clear recognition of the diagnostic importance of the latter reaction before it was made manifest by the work of others. He, however, directed attention to the diagnostic employment of serum with his reaction, and his discoveries formed the basis for the later work on the agglutinative reaction and, therefore, they occupy an important position in the history of serum diagnosis.

In 1895, Bordet, in studying the conditions of production of the Pfeiffer phenomenon outside of the animal body, noted that if a small quantity of immune serum be added to a suspension in salt solution or bouillon of cholera spirilla these lose their motility and become agglomerated. The significance of Bordet's observation is that he was the first to dilute the serum. His interest, however, was chiefly in the determination of the conditions causing the disintegration of the spirilla by cholera serum, and it cannot be said that, at this time, Bordet had a clear perception of the importance and general significance of the agglutinative reaction.

January 3, 1896, Durham presented to the Royal Society a paper giving the results of investigations in Gruber's laboratory in Vienna. This communication embodies the first thorough and systematic study of the agglutinative and immobilizing properties of immune serum outside of the animal body. In this and the rapidly following papers of Gruber and Durham the real importance and general characters of this reaction with immune serum were for the first time made clear. The macroscopic and microscopic tests, the importance of dilutions, quantitative estimations of agglutinative power, the value of the test for the differentiation of bacterial species and for the determination of a previous attack of cholera or typhoid fever, and many other details were described.

It seems but a small step to determine whether a reaction which had been demonstrated to characterize the serum of animals and human beings which have recovered from an infection may not also be present during the period of infection, but upon this step depended the applicability of the reaction as a method of clinical diagnosis. It was Widal who took it and thereby made available for the diagnosis of an infection a reaction which had previously been thoroughly worked out by Gruber and his collaborators so far as immune serum is concerned.[3] Widal's first communication was presented to the Société Médicale des Hôpitaux on June 26, 1896. This first paper has been followed by numerous important contributions by Widal and others to the same subject.[4]

Nature of the Agglutinative Property and Reaction.—As the result of infection with many bacteria or of intoxication with their products, the blood, even when highly diluted, acquires the property of causing loss motility and clumping together of the specific bacteria concerned in the infection or intoxication. The clumping is called by Gruber agglutination, and is attributed by him to the presence of substances to which he has given the name agglutinins. He supposes that these agglutinins make the gelatinous capsules of the bacteria swell up and thereby stick the bacteria together. Although there is no proof of this theory, the names "agglutination," to designate the phenomenon, and "agglutinins," for the supposed substances causing it, have been widely adopted.

In the case of motile living bacteria two phenomena characterize the complete reaction, paralysis or immobilization of the bacteria and clumping. Usually these two phenomena go hand in hand, but sometimes there is loss of motility with little clumping or clumping without much cessation of motion, so that the opinion has been expressed that the paralyzing and the agglutinative substances are not identical. The more common deviation from the usual course of the reaction is the occurrence of clumping, with partial preservation of motility.

The agglutinative serum reaction appears to be of wide, although not universal, application, both for motile and nonmotile pathogenic bacteria, having been demonstrated for typhoid, Asiatic cholera, pneumococcus infection, tetanus, pyocyaneus disease, glanders, hog cholera, Malta fever, colon infection, proteus infection, psittacosis, and several other infections.

The change in the blood upon which the reaction depends is doubtless a specific one in the same sense as are the antitoxic, lysogenic, and other specific alterations caused by the action of definite bacteria or their products. It is upon this specificity that the diagnostic value of the reaction is based. It is true that, as in the case of the antitoxic, lysogenic and other protective modifications of the fluids of the body, the normal blood may possess in some degree the same property, so that the specific character of the change may not be apparent without resorting to considerable dilution of the blood or serum. Normal blood may agglutinate, to some degree, not only the typhoid bacillus but various other bacteria. The specificity of this change resulting from infection with a given microorganism is made apparent by increase of the agglutinative power of the serum only for that microorganism, or to some extent also for closely allied microorganisms. The increase of reaction in some degree with closely allied bacteria, does not militate against the specificity of the change, for it is only an expression of the natural affinities between varieties and races of organisms, as between the typhoid bacillus and the bacillus of psittacosis, or between the cholera spirillum and certain other spirilla, or between the varieties of proteus bacilli or of colon bacilli. The development of the specific agglutinative properties of the blood in typhoid fever affords additional proof, if any were needed, that the bacillus typhosus is the specific cause of this disease.

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[3] It appears that Grünebaum, working in Gruber's laboratory, had before Widal's first publication determined the agglutinative property of the blood serum during the period of typhoid infection, but the results of his investigations were not published until after several papers by Widal and others had appeared.

[4] A summary of their own work on serum diagnosis, as well as that of others, with references to literature, is presented in a recent elaborate paper by Widal and Sicard in the Annales de l'Institut Pasteur, 1897, No. 5.

The blood acquires the specific agglutinative power at a variable period, usually within a few days, after the entrance of the pathogenic microorganism or its products. This power tends to increase, but with much irregularity, during the course of the infection, and gradually to diminish and finally to disappear weeks, months, or it may be years after recovery from the infection. Widal lays much emphasis on the reaction being one of infection and not of immunity. Still it is to be noted that by following procedures for raising experimental immunity to great heights, there may be a corresponding rise of agglutinative power, whereby degrees of this power may be attained which are entirely unknown during natural infections. Thus, Widal, by successive inoculations of an ass, has secured typhoid serum with an agglutinative strength of 1 to 43,000, and Salimbeni has obtained, experimentally, cholera serum agglutinating in a dilution of 1 to 50,000. Gruber speaks even of immune sera which agglutinated distinctly in a dilution of 1 to 500,000. There is, however, no necessary correspondence between the height of immunity and that of agglutination; especially may the latter lessen or disappear when the former is preserved. In the light of the experimental results, and for other reasons, it seems to me somewhat misleading to designate the reaction as merely one of the period of infection.

We are not informed as to the nature of the relationship between the agglutinative and the protective properties of the blood. Gruber has based a new theory of immunity on the agglutinative reaction, but this theory is opposed by many facts and cannot be accepted. The agglutinative property has been shown to be distinct from the bactericidal, lysogenic, antitoxic and other known protective properties of the blood. At present we have no satisfactory evidence that the agglutinative reaction is concerned in any of the defensive mechanisms of the body. According to Salimbeni, whose results, however, are not in entire accord with those of Durham, agglutination of bacteria does not take place within the animal body, although it has been demonstrated that the living blood plasma possesses the agglutinative property.

We do not know the origin or nature of the so-called specific agglutinating substance, save that in some way it results from the activities of bacteria or their products within the living body. Gruber believes that it is derived from the bodies of the bacteria through the agency of the cells. Bordet considers that it is secreted by leucocytes. Experiments of Widal and Sicard, and of Achard and Bensaude, indicate that it is not secreted by leucocytes outside of the living body, but there is nothing which shows that it may not be formed by cells within the body.

The agglutinative substance is generally believed to be a proteid, as it is precipitated from blood plasma with fibrinogen and globulin, and from milk with lactoglobulin and casin, but it is possible that it is simply mechanically retained by these albuminous precipitates. In typhoid fever it may be absent from albuminous urine and may be present in urine which gives no reaction for albumin (Widal). It behaves like an albuminous substance as regards filtration and dialysis.

The question whether it is strictly proper to speak of agglutinative, bacteriolytic, antitoxic, substances in the blood is a legitimate one. They have never been isolated as chemical substances. Behring has expressed the opinion that isolation of antitoxin will never be accomplished, for, in his opinion, it is a force pertaining to highly organized material, and there is no more possibility of separating it as a substance than of isolating the magnetic force from an iron magnet. In all probability these various properties, agglutinative, bacteriolytic, antitoxic, belong to the same general category, and we may look upon the agglutinative property also as a physical one which proteid substance may acquire as the result of the activities of bacteria or their products and no more separable in the form of a chemical substance than is electricity.

The agglutinative property is quite resistant to injurious agencies. It survives desiccation of the blood or serum for months, and may persist for months in blood serum, even when this is seriously contaminated with microorganisms. It is not destroyed by sunlight, unless overheated. It is weakened by prolonged heating at 60 degrees C., and is annulled by heating for ten minutes at 75 to 80 degrees C.

The agglutinative property in typhoid fever is present in maximum amount in the blood, being somewhat greater in the blood plasma than in the blood serum. It is found in blister serum in essentially the same strength as in blood serum. In other fluids, as the pleural, peritoneal, pericardial, inflammatory and oedematous, it is in smaller and variable amount. In milk and colostrum it is present in marked degree. It is weak and inconstant in the urine, bile and aqueous humor. It has been found in tears naturally secreted, but is said to be absent from those provoked by irritants. It may be present in typhoid stools (Block). It has not been found in the cerebrospinal fluid or in the fluid in the seminal vesicles. It has not been positively determined whether the distribution of the agglutinative property in the various humors of the body outside of the blood, can be explained wholly by processes of filtration and diffusion from the blood plasma, although these are doubtless the main factors.

The agglutinative reaction may or may not be obtained with the blood of a foetus or new born infant of a mother with typhoid fever. Chambrelent and Saint-Philippe consider that the presence or absence of the reaction in the foetus depends on whether or not the typhoid bacilli break through the placental barrier from mother to foetus and cause infection of the latter. Further investigations are needed to determine this point and thus to decide whether the agglutinative property is passively or actively acquired by the foetus. For it has been shown that this property, like immunity, not only may be actively acquired as the result of infection or intoxication, but may be passively transmitted by injection of agglutinative serum, the reaction in the latter case appearing promptly without symptoms of infection, being relatively slight and disappearing after a short time.

Courmont thinks that the development of typhoid bacilli in a fluid robs it of agglutinative power. Thus he has found that the vegetation of the bacilli in typhoid serum deprives it, in a few days, of the agglutinative property, and that blood obtained, postmortem, from the spleen, liver and mesenteric glands, organs in which the typhoid bacilli are especially abundant, is poorer in agglutinative power than that from other parts. Ménétrier found in a case of typhoid fever that the pleural exudate, which usually gives the agglutinative reaction, did not do so, and that it contained typhoid bacilli in large number. It has been suggested that these observations may shed light on the exceptional cases of typhoid fever with absence of the specific serum reaction. Flexner has shown that, occasionally, the typhoid bacilli develop in the blood in such large numbers as to produce a genuine typhoid septicaemia. Still these observations, interesting as they are and deserving further investigation, must be interpreted with caution, for Widal has found a purulent exudate from an immunized ass, swarming with typhoid bacilli, to present, even after fifteen months' preservation, an agglutinative power of 1 to 13,000, the power of the blood serum of the same animal kept for the same length of time being 1 to 14,000.

We have no satisfactory explanation of the production of the phenomenon of agglutination by specific serum. Gruber's explanation already mentioned has received no confirmation. The phenomenon occurs with nonmotile as well as with motile bacteria, with dead as well as with living organisms. Typhoid bacilli killed by formol in weak solution, or by heating for five minutes at a temperature of 56 degrees C., are about as sensitive to the reaction as are living bacteria, and retain their agglutinability for a long period (Widal). The phenomenon, therefore, is a physical rather than a vital one, although probably dependent in some way on the protoplasmic constitution of the bacterial cell. Salimbeni, as has been stated, found that the phenomenon does not occur in the living body of immunized animals. He also found that the presence of atmospheric air greatly favors the reaction, it requiring much more concentrated serum and a longer time to bring about the phenomenon in a vacuum than when the fluid is exposed to the air. Widal has shown that other physical conditions, particularly contact with objects, such as the surface of slide or cover-glass, and partial evaporation, favor the production of the reaction.

Agglutinative serum is often likewise bactericidal and bacteriolytic, but the agglutinative reaction is independent of the bactericidal and is manifest with dilutions of the serum which annul the bactericidal power. In such dilutions the agglutinated and immobilized bacteria are not altered morphologically, or in staining properties, or in pathogenic power, or in any other way, so far as has been determined. Whether the temporary inhibition of bacterial growth in agglutinative serum is dependent on the agglutinative or on some other property of the serum is not known.

Methods of Making the Serum Test for Typhoid Fever.—Widal, in his first communication, described both the slow or macroscopic and the quick or microscopic methods. For each of these he recommended a dilution of one part of blood or blood serum to ten parts of the fluid containing the culture. The macroscopic method consists in adding the blood or serum to be tested either to a young bouillon culture of the typhoid bacillus or to sterile bouillon which is then at once inoculated with the bacillus. In the former case the reaction with typhoid serum appears usually within two or three hours and consists in clarification of the previously turbid fluid, and the formation of a clumpy sediment composed of accumulated bacilli. In the latter case the tube is placed in the incubator and within fifteen hours the reaction is manifested by growth of the bacilli in the form of a sediment at the bottom of the tube, the fluid remaining nearly or quite clear.

The microscopic test, to which Widal gave the preference, is made by mixing the blood or serum with a young bouillon culture or with a suspension in bouillon or salt solution of a fresh growth of the typhoid bacillus and examining a drop or two of the mixture at once under the microscope. With a dilution of 1 to 10 this microscopic typhoid reaction appears, as a rule, immediately or within a few minutes, and is evidenced by loss of motility and by clumping of the bacilli into masses of various sizes and shapes.

Widal obtained the blood either with sterilized hypodermic syringe from a vein of the arm, or by pricking the finger. It may also be conveniently obtained by pricking the lobule of the ear. A few drops of blood suffice for collecting the necessary amount of serum; indeed a single drop will do for the reaction. The blood may be collected in a small test tube where, usually in a few minutes, it clots. The separation of the serum may be facilitated by passing a sterilized platinum needle between the glass and the clot, or by the centrifuge, or the blood may be collected and allowed to clot in a slanted tube, which is then placed upright, the separated serum trickling to the bottom.

In a communication made July 31, 1896, Widal said that results equaling those with blood serum can be obtained with blister serum, and this procedure has been employed with much satisfaction by the Health Department of New York City.

At the same time Widal called attention to the preservation of the agglutinative property in dried blood and serum. Wyatt Johnston deserves the credit of developing the test with dried blood and for introducing the method of serum diagnosis into the work of municipal laboratories. The dried blood method, which has been used far more extensively in Canada and this country than in Europe, possesses certain manifest advantages, especially ease of collection, freedom from subsequent contamination and readiness of transportation, and it has given excellent results in the hands of Johnston and others. The principal objection, and this is of considerable importance when precise results are desired, is the difficulty of obtaining accurate quantitative dilutions with the use of dried blood.

Several observers, including Breuer, Haedke, Du Mesnil de Rochemont, Scheffer, have expressed the opinion that the macroscopic method is more trustworthy than the microscopic. This I believe to be an error and to be due to unfamiliarity with all of the conditions essential for the accurate employment of the microscopic test. The latter is more delicate, prompt and precise than the macroscopic reaction, and requires less care in respect to accidental contamination.

A year's experience with the method of serum diagnosis of typhoid fever has led to a general consensus of opinion as to its great value. It has, however, been recognized that certain precautions in the application of the test are necessary in order to avoid mistakes. Numerous modifications of the original methods have been suggested, the most important relating to quantative determinations. In considering the value and practical utility of such modifications of the test, several points should be borne in mind. Practically all of the methods recommended by competent investigators have given good results in the great majority of cases. For clinical purposes it is desirable that neither the method of obtaining and collecting the blood nor that of conducting the test should be made more difficult and complicated than is absolutely necessary. Methods which may be essential for exact scientific work, where every possible source of fallacy is to be avoided, may not be the best for the routine examinations of a clinical or a municipal laboratory. Where absolute accuracy is not obtainable it is upon the whole better that the method should err on the side of now and then including a nontyphoid case than in excluding cases of genuine typhoid fever. With due allowance for such considerations as these, we must welcome all efforts to give greater precision to the methods of serum diagnosis and to determine the capabilities of these methods and their possible sources of error. In exact quantitative work with serum test the most important points to be considered are the characters of the culture, the dilution of the serum, the time limits, the criteria of the reaction and certain physical conditions influencing the reaction.

Characters of the Culture.—There has been considerable difference of opinion as to whether cultures of the typhoid bacillus obtained from different sources are equally sensitive to the agglutinative reaction. Widal, Durham, Stern and C. Fraenkel, who have all had large experience with different cultures, have found only unimportant and inconstant differences in susceptibility to the reaction. The fact determined by Pfeiffer that the less virulent the culture, the greater the sensitiveness to the lysogenic reaction (Pfeiffer's phenomenon), seems to have been considered by many without sufficient investigation to be equally applicable to the agglutinative reaction. Kolle, without however presenting sufficient evidence, emphasizes the greater susceptibility of cultures with weakened virulence to agglutination. The most satisfactory evidence on this point is furnished by Kühnau, who made a careful comparative study of the behavior with the serum test of a nonvirulent and a virulent typhoid culture, and found the former to react much more intensely with normal and typhoid sera. He, therefore, lays stress on consideration of the virulence of the culture in quantitative work with the serum test. In view of the conflict of opinion further investigations upon this question are needed.

It cannot be doubted that several observers have had to do with typhoid cultures which presented distinct differences in susceptibility to the agglutinative reaction. Especially worthy of consideration, although not wholly in accordance with some results of others, are the observations of Johnston and McTaggart, confirmed by Appel and Thornbury, that solutions of dried blood are more potent than serum in agglutinative power, although not in paralytic effect, and that such solutions from nontyphoid cases are prone to give partial (pseudo) reactions with frequently transplanted typhoid cultures, whereas this difficulty is largely overcome by using fresh cultures planted from stock cultures a month old. Hence they strongly recommend for the dried blood method cultures of the latter character. They, as well as other writers, likewise emphasize the importance of considering the composition of the culture medium, which should be favorable to vigorous growth and not too strongly alkaline.

Only young cultures should be used, preferably not over twelve to eighteen hours old, if grown in the incubator. Older room cultures can be used. Old cultures agglutinate more readily than young ones. Either bouillon cultures or suspensions in bouillon from solid cultures may be employed. There is no difficulty in securing uniform suspensions of isolated, actively motile typhoid bacilli, especially from young cultures on dried out agar. In every case it is of prime importance to make a control examination of a drop from the same part of the culture or suspension which is used for the test and at the time of making the test in order to be sure that there are no preexisting clumps, that the bacilli are actively motile, and that the culture is not contaminated.

Stern suggested that the concentration of the suspension, that is the number of bacilli in it, may be a factor meriting consideration, and Kühnau and Block have shown that this is the case. Weak suspensions are more readily agglutinated and paralyzed than stronger ones. Hence, Kühnau recommends the use of suspensions of known concentration, which can be approximately secured without much difficulty. He uses a suspension in bouillon of a fifteen-hour virulent agar culture (grown in the incubator) containing about one hundred and twenty million bacteria in a cubic centimeter.

Dilution of the Serum.—Inasmuch as normal and nontyphoid blood may possess distinct agglutinative property, especial importance is attached to dilution of the serum, in order to avoid mistaking the normal reaction for one of typhoid fever. The opinion has been widely expressed that the dilution recommended by Widal, 1 to 10, is too low, and that a dilution should be used which is not known ever to give a reaction with nontyphoid blood. The fixation of the upper limit of such dilution has been placed gradually higher and higher, thus by du Mesnil at 1 to 25, by Kolle 1 to 30, by Grünbaum 1 to 33, by Stern 1 to 40, by Kühnau 1 to 50. Even if it should be admitted that a reaction in nontyphoid cases with these higher dilutions is ever of such a character as might mislead an experienced observer, its occurrence is, according to most observers, very exceptional.

The question arises whether the adoption of a dilution of say 1 to 50 as the standard, would result in the exclusion of genuine typhoid cases from the diagnosis. Widal divides the typhoid cases in which he has measured the agglutinative power of the blood into five groups: *a,* those with very weak power, less than 1 to 100 (four cases); *b,* with weak power, between 1 to 100 and 1 to 200 (nine cases); *c,* with medium or average power, from 1 to 200 to 1 to 500 (eight cases); *d,* with high power, from 1 to 500 to 1 to 2,000 (nine cases); and *e,* with very intense power, exceeding 1 to 5,000 (three cases). In only one case did the agglutinative power not rise over 1 to 40, it being 1 to 30 on the twentieth, and 1 to 40 on the twenty-second day of the disease. In one case, Widal found the strength to be 1 to 12,000. In nineteen cases measured by Stern, the agglutinating strength was never less than 1 to 50. C. Fraenkel found the average to lie between 1 to 100 and 1 to 200, sometimes reaching 1 to 5,000. Out of seven cases Kühnau found two in which the serum was active only in dilutions less than 1 to 50, it being 1 to 30 in one, and 1 to 20 in the other case, but in both he made a positive diagnosis of typhoid fever in consequence of disparity of the action of the serum on the colon bacillus and the typhoid bacillus. From the observations thus far reported, although they are insufficient in number for definite conclusions, there would seem to be only small liability of failure to recognize genuine typhoid cases by resorting to dilutions of 1 to 40 or 1 to 50, but unquestionably a few cases would escape recognition, and for this reason lower dilutions should also be used, and if those between 1 to 10 and 1 to 50 give decided reaction there should be, at least, suspicion of typhoid fever.

It is not, therefore, to be recommended that one should make the test with only high dilution, such as 1 to 50. The negative result of a preliminary test with equal parts serum and culture suffices to exclude typhoid reaction. The examination, if positive, may then be made with low dilution of the serum and for this Widal's recommendation of 1 to 10 or 1 to 15 may be well adopted. If with this dilution the microscopic reaction is complete and almost immediate, as is often the case, there is practically no risk in making a positive diagnosis. But for absolute certainty and above all in cases where the result of the reaction is not prompt, complete and unmistakable, higher dilutions should be employed; if the amount of serum permits only one such, it may be 1 to 50, but preferably intermediate dilutions should also be made, and it is desirable, if not absolutely necessary, to try dilutions higher than 1 to 50. For making the dilutions there are various simple technical procedures, which involve but little expenditure of time and labor and only small quantities of serum, as for example, that recommended by C. Fraenkel. An accurate fixation of the upper limit of agglutinative power is often tedious and not generally necessary in diagnostic work. A positive diagnosis of typhoid fever, based exclusively on the test with a low dilution, in a case which subsequently proves not to be typhoid should not be considered as in any way invalidating the results of an accurate employment of the method of serum diagnosis.

It is self-evident that the employment of varying degrees of dilution of the serum, without at the same time taking into consideration other factors which influence the reaction, has little sense and does not constitute, in itself alone, an accurate method of mensuration of agglutinative power.

Time Limits.—As the rapidity with which the reaction appears and progresses generally varies, other things being equal, according to the agglutinative strength of the blood it is evident that methods of exact mensuration of this strength must take into consideration the length of time required for the development of the reaction after the addition of the serum. Many writers have not paid much attention to this point. Stern has proposed that a limit of two hours be adopted as an arbitrary standard for the microscopic reaction, and Widal has accepted this proposal. With this unit, an agglutinative power fixed at 1 to 500 means that 1 part of serum added to 500 parts of the fluid containing the culture, agglutinates and paralyzes the bacilli within two hours, although a higher dilution may give a decided reaction in six or eight hours. The optimum effect is, according to Stern, not attained before the lapse of six or eight hours.

By varying the time limits, results obtained by lower dilutions may be roughly comparable with those by higher dilutions. Thus, for diagnostic purposes, a fifteen minute time limit for dilutions of 1 to 10 may be adopted and a two hour time limit for dilutions of 1 to 50 or higher, but it should be understood that in all doubtful cases quantitative determinations by varying the dilution should be employed.

Criteria of the Reaction.—Some writers have proposed to make either the cessation of motility or the clumping the essential criterion of the reaction. Thus Stern selects the clumping and Kühnau the paralysis of motion. In my judgment both phenomena enter equally into the reaction and deserve equal consideration, so that a reaction is not to be considered complete and satisfactory unless the bacilli are both clumped and rendered immobile. Partial reactions in which one or the other characteristic is lacking may warrant suspicions and lead to further examination, but they should not be made the basis of positive diagnosis. For this reason the use of killed cultures, as suggested by Widal, while it may have a limited field of application, cannot supplant the ordinary method. As already stated, the microscopic reaction is to be preferred to either of the macroscopic methods, although the latter afford striking objects for demonstration. With low dilutions bactericidal and lysogenic phenomena are common, but they do not pertain to the agglutinative reaction itself.

Certain Physical Conditions Influencing the Reaction.—As already mentioned Salimbeni has shown that free exposure to the air favors the reaction and Widal has pointed out that partial evaporation at the edge of the cover-glass and contact of the specimen with slide and cover-glass are also favoring conditions. Hence, the conditions for the reaction are not exactly the same with the serum bouillon mixture in thin layer beneath the cover-glass on an ordinary slide, as in a thick layer, or in a sealed drop culture on a hollow-ground slide, or in a column of fluid in a test tube, or in a moist chamber. The presence of fibrinous masses, granules and material foreign to blood serum may perhaps explain in part the greater frequency of partial reactions with normal blood when the dried blood method is used than when the serum method is employed. The temperature of the incubator, by favoring evaporation and in other ways, accelerates the reaction. For exact quantitative work these various physical conditions need consideration and further investigation. Widal prefers the use of ordinary slides to that of hollow-ground slides, and does not advise keeping the specimen in the incubator. It is not to be supposed that the diagnostic use of the serum test generally hinges on such delicate points as these, but they are among the points to be considered in the explanation of certain irregularities in the results of the test, in comparing the results of different workers, and in mensuration of agglutinative power, especially with high dilutions.

Reactions with the Colon Bacillus.—Statements of different writers as to the occurrence of the agglutination of the colon bacillus with normal and typhoid sera are not harmonious. Widal and Courmont find that all human sera, whether normal or typhoid, have a slight agglutinating action on the colon bacillus in dilution of 1 to 10, whereas normal sera have only exceptionally any such action on the typhoid bacillus in this dilution. Many observers have noted some agglutination of colon bacilli with typhoid serum, although the reaction is much less intense than with the typhoid bacillus. Vedel found, in a case with symptoms of typhoid fever but without the typhoid serum reaction, marked colon reaction, and he interpreted the case as one of colon infection simulating typhoid. He is not, however, inclined to attach much diagnostic importance to the colon reaction, as he found that it might be well marked both with normal and typhoid blood. Johnston and McTaggart found genuine colon reactions with typhoid blood to be rare, provided the typhoid reaction was well marked. In several cases, however, where the symptoms suggested typhoid but the typhoid serum reaction was absent, they found marked colon reaction. They are inclined, therefore, to attach diagnostic importance to the latter reaction. The colon cases were mild and of shorter duration than ordinary typhoid fever.

Kühnau makes use of the colon reaction to assist in the diagnosis of typhoid fever in doubtful cases. He finds that normal serum reacts in the same way with both colon and typhoid bacilli, whereas typhoid serum, even when of weak specific power, reacts much more intensely with the typhoid bacillus than with the colon bacillus. By availing himself of this unequal action of typhoid serum upon the two species of bacteria, he felt justified in making the diagnosis of typhoid fever when the agglutinative power of the serum did not exceed 1 to 20. Kühnau's suggestion is interesting, but further investigations are needed to determine its value.

We must also await further studies before Johnston and McTaggart's highly suggestive views as to the existence of colon infections simulating typhoid and capable of diagnosis by the serum reaction with the colon bacillus can be accepted.

As was first pointed out by me in 1890, the colon bacillus is an extremely common secondary invader of the body in all sorts of conditions, particularly those with lesions of the intestine. It can very frequently be found in internal organs outside of the intestine in typhoid fever, if careful search is made. We have no satisfactory proof that it produces either symptoms or lesions in most of these cases, and one would expect more common and intense serum reactions with *Bacillus coli* in typhoid fever, if the organism was engaged in pathogenic work. The writer has repeatedly taken occasion to protest against what seem to him unwarranted inferences as to the pathogenic significance of the mere detection of the colon bacillus in the internal organs at autopsies, although there can be no question that under certain conditions this bacillus may be pathogenic for man.

As the colon group of bacilli contains numerous races, some approaching the typhoid bacillus closely, it is to be expected that they will vary markedly in their sensitiveness to agglutination with different sera.

Durham found that typhoid immune serum in no instance produced any agglutinative reaction with ten different specimens of *Bacillus coli* obtained from various sources. Colon immune serum reacted on its own race of bacilli exactly like typhoid serum on typhoid bacilli, but it did not react with all races of colon bacilli, a graduated series of effects being observed with different specimens of these bacilli. Rodet, however, whose results are reported in much less detail than those of Durham, found a certain degree of reciprocal action between colon and typhoid immune sera and their respective bacteria.

Date of Appearance and Disappearance of the Typhoid Serum Reaction. Absence of Reaction.—The presence of the specific agglutinative reaction can usually be counted on by the end of the first or the beginning of the second week of typhoid fever. It may appear as early as the second day of the disease (Johnston and McTaggart, C. Fraenkel), but this is very exceptional. It may here be noted that the determination of the exact day of the disease, often so gradual and insidious in its development as typhoid fever, must frequently be more or less arbitrary, and will vary according to the case and with different observers. Sometimes the first appearance of the reaction is delayed, exceptionally until the end of the second or into the third week, or even later. There are rare cases in which the reaction is missed during the first attack and makes its appearance in the relapse (Breuer, Thoinot, Biggs and Park, and others). It has even been missed until the first days of convalescence. Blumenthal relates an interesting case in which the reaction was absent during the fever, tests being made on the twelfth and twenty-first days with serum dilutions of 1 to 10, but it was found with dilutions of 1 to 100 two days after the beginning of apyrexia. Achard likewise once found the reaction only during convalescence. We have not at present a sufficient number of accurate data to furnish definite figures as to the frequency of these delayed reactions, but their occurrence undoubtedly constitutes a defect in the method of serum diagnosis of some importance. A negative result of the test does not exclude the diagnosis of typhoid fever. The probability against this diagnosis is the greater, the later the period of the fever in which the negative result is obtained and the oftener the examinations are repeated. As regards the interpretation of negative reactions, the serum test does not differ from other bacteriological diagnostic tests, that for the tubercle bacillus for instance.

There are authentic cases of typhoid fever in which repeated examinations of the blood during the course of the disease and its convalescence failed to reveal the specific agglutinative reaction, even with serum dilutions of 1 to 10. We cannot at present say what percentage of the total number they make. Widal and Sicard found absence of the reaction in only one out of 163 cases of typhoid fever examined by them. In this negative case, in which the diagnosis was confirmed by cultivation of typhoid bacilli obtained by hypodermic puncture of the spleen, the reaction was absent during the fever, the apyrexia, the relapse and the convalesence. Of 116 cases of typhoid fever examined by Courmont, the reaction appeared in all, being delayed after the eighth day in only five. Of 70 cases examined by Chantemesse, it was present in all. Of 129 cases examined by Johnston and McTaggart, if a few cases examined only late in convalescence, or at a very early stage without reexamination, be excluded, the reaction was missed in only one. In many reports cases, believed to be typhoid, are recorded as giving negative reaction when only one examination was made, this being sometimes early in the disease. Such cases doubtless belong mainly to the group with delayed reaction. The importance of repeated examinations is illustrated by such observations as Stern's, in which the test was negative at the end of the second week and positive two days later; of Widal's, negative on the tenth, positive on the twenty-second day, and several others of similar purport.

The agglutinative power of the blood tends to increase during the progress of the fever, but there are exceptions, and in general the intensity of the reaction is subject to irregularities and oscillations, which may be notable from day to day. There may be marked sudden rise or fall of reactive power. While weak reactions are more common in mild cases, there is no definite correlation between premature or delayed development or the intensity of the reaction and the gravity of the disease. The persistence of high agglutinative power, for example 1 to 2,000, after subsidence of the fever, does not prevent relapses.

In the majority of cases the specific agglutinative power of the blood diminishes in the first weeks or months after cessation of the fever and disappears within a year. Exceptionally it may vanish as early as eight or ten days after the fever. Widal and Sicard noted its disappearance on the eighteenth and twenty-fourth days, Breuer on the seventeenth and twenty-fifth days, E. Fraenkel on the twenty-eighth day after defervescence, etc. Disappearances at such early dates as these are, however, not the rule. According to Courmont's experience, the serum reaction disappears in children most frequently during the course of the first two months, and in adults toward the fifth and sixth months, although it is not uncommon for it to continue a year. The specific reaction may, however, persist for years, perhaps indefinitely. Of forty cases which had typhoid fever at least a year before examination, Widal and Sicard found the agglutinative reaction, either marked or slight, in eleven; after one and one-half year, one case, reaction weak; after two years, one case, reaction weak; after three years, two cases, in one marked, in the other weak; after six years, one case, reaction 1 to 10; after seven years, one case, reaction marked; after eight years, one case, reaction 1 to 1,800; after nine years, three cases, one marked, one 1 to 40, one 1 to 30; after twenty-six years, one case, 1 to 30. Kühnau found after one year, two cases with reaction of 1 to 80; after two years, one case, reaction 1 to 60; after seven years, one case reaction 1 to 60. All others of a series examined (total number not stated) showed negative reaction after one year. It was observed by Widal and Sicard that in contrast to the reactions during infection and for the first weeks after defervescence, those of long standing showed no notable fluctuations in intensity during the periods of examination, extending sometimes over several weeks.

The persistence of the specific reactions after typhoid fever is of importance from two points of view, retrospective diagnosis and interpretation of the diagnostic significance of the reaction during a febrile infection. It is sometimes of interest and practical importance to determine that an individual has previously had typhoid fever. Thus Courmont was able by the serum diagnosis to determine that a patient with multiple neuritis, supposed to follow an attack of dysentery, was in reality convalescent for a month and a half from typhoid fever, and Achard recognized the real nature of an attack of osteomyelitis in a patient who had had typhoid fever a year before (cited from Widal and Sicard).

Many writers have called attention to the evident possibility of a mistake in diagnosis when the serum reaction is found in a person with febrile infection who has recovered from typhoid fever, although it does not appear that any serious difficulty has been encountered thus far from this source of error. It, however, indicates the importance of obtaining a careful history of the patient, not only as regards recognized typhoid fever, but as to attacks interpreted as dysentery, gastric fever, appendicitis, malaria, etc. An observation reported by Stern indicates that the specific typhoid reaction may be acquired even without manifest illness. He suggests that careful quantitative estimations of agglutinative power may restrict the possibility of error in diagnosis arising from long persistent reactions, as increase or diminution in the course of the fever or of the convalescence would speak for fresh infection. Even if the fullest possible allowance be made for this source of error, it applies to so small a number of cases that the value of the method is not seriously impaired.

Presence of the Reaction in Non-Typhoid Cases.—The blood of many hundred persons, either healthy or affected with diseases other than typhoid, has been tested for the specific typhoid reaction, and it can now be asserted that a serum reaction which an experienced observer using accurate methods would consider characteristic of typhoid fever, is to be found only most exceptionally in those who have not had typhoid infection.

Several observers, especially the Germans, find that by adherence to Widal's original directions mistakes may occur, but that these can be avoided by attention to quantitative determinations, especially dilution of the serum, time limits, and characters of the culture used for the test. The most remarkable observations on this point come from Breslau and are reported by Stern and Kühnau. Stern examined the blood serum of seventy persons not suffering from typhoid fever and, according to their statements, never having had typhoid fever. In twenty of these the serum had an agglutinative strength of 1 to 10, in five a strength of 1 to 20, and in two a trace of reaction was obtained with dilutions of 1 to 30. In none of these cases did he find any reaction with dilutions of 1 to 40. Of more than fifty similar cases examined by Kühnau, in forty-one no reaction was obtained with dilutions higher than 1 to 5. In eight agglutination was observed with dilutions of 1 to 10, up to 1 to 20; in four with 1 to 30; in three with 1 to 35, up to 1 to 40; and in one even with 1 to 50.

In judging these results, apparently so divergent from those of Widal and nearly all others, it is to be noted that both Stern and Kühnau used the microscopic reaction, made two hours the time limit, even for the lowest dilutions, kept the specimens in the thermostat, and that Stern regarded the clumping and Kühnau the paralysis of motion as the criterion of the reaction, thus apparently recording as genuine what many others would consider partial or pseudoreactions. It is clear that those who interpret only complete reactions occurring within fifteen to thirty minutes at room temperature as genuine reactions, would not be likely to obtain any such results as those reported by Stern and Kühnau. Nevertheless, it must be admitted that even partial and late reactions are unwelcome and disturbing, even if an observer thinks that his skill and experience will enable him to avoid mistakes from their occurrence. It may also well be, as suggested by C. Fraenkel, that Stern's and Kühnau's normal and nontyphoid cases include not a few who had recovered from unrecognized typhoid infection, and this supposition is the more probable in consequence of the prevalence of typhoid fever in Breslau. Stern himself calls attention to the liability of failure to recognize certain mild cases.

There have been a few cases reported in which the diagnosis of typhoid fever was made on the basis of the serum reaction, but which the authors, from subsequent developments, considered to be free from typhoid infection. It has been claimed, therefore, that positive serum reactions are not a sure sign of typhoid fever, although no one claims that the chances of error from this source are more than slight. The cases reported by Achard and Bensaude, Jez, Farrand, du Mesnil and van Ordt have given rise to the most discussion. For what seem to me justifiable criticisms of these reports, I would refer especially to the papers of Widal and Stern, and I will limit my remarks to some general statements concerning such alleged failures of the serum test.

In the first place, as the matter now stands, positive reactions obtainable only with dilutions lower than 1 to 50, possibly than 1 to 60, especially if the reaction is partial and late in appearance, are not certain diagnostic signs of typhoid fever. In most of the cases just referred to no exact quantitative estimations of the agglutinative strength of the serum were made, and hitherto it has not been shown that the reaction ever occurs with nontyphoid serum in dilutions exceeding 1 to 50, with observance of other quantitative points which I have already considered. With the limited number of observations, however, which we now possess, we cannot, of course, say but that such cases will be found in the future.

In the second place, infection with the typhoid bacillus cannot be positively excluded either on clinical grounds alone or by anatomical examinations at the postmortem table. Infections with the typhoid bacillus occur without any characteristic anatomical lesions. There may be entire absence of ulcers or other lesions of the intestine. We have recently had at The Johns Hopkins Hospital a case with positive serum reaction, from which Dr. Flexner cultivated, in large number, typical typhoid bacilli from the gall-bladder although there was no previous history of typhoid fever, and there were no intestinal lesions. In 1891 I called attention to the favorable conditions offered by the bile for the prolonged survival of the typhoid bacillus. In one case I was able to demonstrate large numbers of typhoid bacilli in the bile of the rabbit, 128 days after intravenous injection of 0.5 c. c. of a bouillon culture.[5] Pick has reported a case with marked positive serum reaction in which at the autopsy no typhoid intestinal lesions and no swelling of the spleen were found, but bacteriological examination showed the presence of typhoid bacilli, not however in the spleen. Guinon and Meunier's case is instructive. During life the symptoms were those of acute miliary tuberculosis and typhoid fever combined. Serum reaction was positive. At autopsy the lesions appeared to be only those of acute miliary tuberculosis, small ulcers in the intestine being typically tubercular in aspect. Typhoid bacilli, however, were cultivated from the spleen and other parts. As both the symptoms and the bacteriological examination indicated that the typhoid infection was in course of disappearance, the case, if examined at a somewhat later period, might readily, as Guinon and Meunier remark, be placed to the dicredit of the positive value of the serum test. We are justified, in the light of such cases as these, in demanding that thorough bacteriological examinations be made before cases which have given during life the characteristic serum reaction, but which do not present at autopsy the anatomical lesions of typhoid fever, be recorded as free from infection with the typhoid bacillus.

In the third place, the difficulty of excluding a previous attack of typhoid fever, after which, as already stated, the specific serum reaction may persist for years, is to be borne in mind.

In conclusion, I would emphasize the following practical points:

1. Experience has demonstrated that the method of serum diagnosis of typhoid fever is of great practical value.

2. The alteration of the blood on which this method is based, is a specific effect of infection or intoxication with the typhoid bacillus.

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[5] Welch: Bulletin of The Johns Hopkins Hospital, August, 1891.

3. The microscopic serum test is to be preferred to the macroscopic methods.

4. Quantitative determinations, relating especially to the culture, the time limits, and the dilution of the serum, are of importance and, at least in doubtful cases, should not be neglected.

5. As the reaction may be delayed or occasionally absent, a negative result of the test does not exclude the diagnosis of typhoid fever. The later in the course of the disease the test is applied, and the oftener the examinations are repeated at intervals, the less is the probability of the existence of typhoid fever.

6. The persistence of the reaction, sometimes for years, after recovery from typhoid fever, is to be borne in mind in interpreting the reaction in febrile conditions. The appearance of the reaction and its increase during the period of observation speak for fresh typhoid infection.

7. The danger of mistakes from positive reactions in nontyphoid cases can be guarded against in nearly all cases.

8. Provision should be made, especially by the establishment and support of municipal or state laboratories, to render generally available to practitioners the serum method of diagnosis, as well as other bacteriologic procedures of similar practical value.

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THE ACTION OF RATTLESNAKE VENOM UPON THE BACTERICIDAL PROPERTIES OF THE BLOOD[1]

The venom was obtained directly from a living rattlesnake in a sterilized saucer, according to the method recommended by Weir Mitchell. This venom was injected in varying doses subcutaneously into rabbits, which died at intervals varying from one-half hour to four hours. Immediately after death the blood was withdrawn by means of a sterilized glass pipette from the right auricle and posterior vena cava, about 6 to 10 c. c. being readily obtainable in this way. At the same time blood was obtained for a control experiment from a healthy rabbit. In some instances the blood of the animal before it was poisoned by the venom was previously tested as to its bactericidal power. After standing twenty-four hours in the refrigerator, 1 to 3 c. c. of the venom serum and of the normal serum were pipetted off into sterilized test tubes and inoculated with the bacteria to be tested. *Bacillus coli communis* and *Bacillus anthracis* were used for this purpose. Roll or plate cultures in gelatine or in agar were made in the usual way to determine the number of bacteria inoculated into the serum and at varying intervals of time up to one week to determine increase or diminution of the bacteria planted in the serum. The experiments showed that the blood serum of rabbits killed with the venom was nearly or entirely devoid of the power of destroying the bacteria used for the experiments, whereas the normal serum was capable in a few hours of destroying thousands of these bacteria.

The bearing of these experiments upon the rapid development of post mortem decomposition following death from snake bites and also upon individual predisposition to the invasion and multiplication of bacteria in the living body was considered.

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[1] Report of remarks of William H. Welch and C. B. Ewing before the Section on Pathology of the Pan-American Medical Congress, Washington, D. C., September 6, 1893. A more detailed account of Dr. Welch's experiments was given by C. B. Ewing before the Association of Military Surgeons, Washington, D. C., 1894, and published subsequently in The Lancet, Lond., 1894, I, 1236-1238.

Tr. Pan-Am. M. Cong., Wash., 1893, I, 354-355.

THE HUXLEY LECTURE ON RECENT STUDIES OF IMMUNITY, WITH SPECIAL REFERENCE TO THEIR BEARING ON PATHOLOGY[1]

Gentlemen.—You will readily believe that with my deep appreciation of the high honor conferred by the invitation to deliver the fourth Huxley lecture there was joined a sense of great embarrassment in being called upon to follow in this office three such leaders of world-wide fame as Sir Michael Foster, Professor Virchow and Lord Lister. But the letter of the Committee of the Charing Cross Hospital Medical School stated that the choice of a successor to these great names was "a tribute of our admiration for the great army of scientific workers on the other side of the Atlantic." While I cannot assume to occupy any other place in this army than that of a soldier in the ranks, I felt that if my acceptance of this invitation could be regarded as in any sense an expression of appreciation by American workers in science of the commendation and good will of our British colleagues, of our large indebtedness to them, of our sense of the common interests, the comradeship and the kinship of the English-speaking peoples on both sides of the ocean, I should not decline, even if summoned to occupy a position of danger.

There was another consideration which I may be permitted here to mention. Through Huxley there is, if not a bond, at least a link, between the Charing Cross Hospital Medical School and The Johns Hopkins University. This lectureship was founded to commemorate the fact that Huxley received his entire medical education at the Charing Cross Hospital Medical School. While throughout America the name of Huxley is held in high honor as that of a great discoverer and interpreter in science, and while the influence which he has exerted upon popular as well as scientific opinion through those messages peculiarly fitted to the needs of English thought is not less there than among his own countrymen, we at The Johns Hopkins University have special reasons to acknowledge our gratitude to him. He crossed the ocean to deliver the principal address at the opening of this university in 1876, and he then gave utterance to ideas concerning university, and especially medical, education which were at the time and have remained an inspiration and a guide to us. Then, too, The Johns Hopkins University owes to Huxley and to Michael Foster the accession to its faculty of my lamented colleague, Newell Martin, who by the introduction and development of the biological methods and conceptions of his teachers gave such new directions and so great an impulse to biological study in America that his own work and that of his pupils started for us a new era in this department.

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[1] Delivered at the opening of the winter session of Charing Cross Hospital Medical School, London, October 1, 1902.

Brit. M. J., 1902, II, 1105-1114.

Science, N. Y. & Lancaster, 1902, n. s., XVI, 804-816; 850-861.

The first Huxley lecturer has made it unnecessary for his successors to dwell upon Huxley's studentship at the Charing Cross Hospital, upon the important influence which this had upon his career, or upon his great services to medical science, although his chief title to fame lies outside of the domain of medicine. I should like, however, to quote a passage, although it must be familiar to you, from Mr. Leonard Huxley's charming "Life and Letters" of his father, which has appeared since the date of Sir Michael Foster's lecture, for it shows that "it was at Charing Cross Hospital where Huxley first felt the influence of daily intercourse with a really able teacher." He says:

> "No doubt it was very largely my own fault, but the only instruction from which I obtained the proper effect of education was that which I received from Mr. Wharton Jones, who was the lecturer on physiology at the Charing Cross School of Medicine. The extent and precision of his knowledge impressed me greatly, and the severe exactness of his method of lecturing was quite to my taste. I do not know that I have ever felt so much respect for anybody as a teacher before or since."

Wharton Jones, who will doubtless be longest remembered as the discoverer of the amoeboid movements of the white blood corpuscles, was an experimental physiologist and pathologist of much originality, and it seems to me that there has not been, even in his own country, so full a recognition of his work as its importance merits.

Before passing to the special theme of this lecture it is fitting that I should pause, if only for a moment, to call to mind with affection and reverence that recently departed great man who honored and delighted you four years ago, and who has conferred such high distinction upon the office of Huxley lecturer. When one considers the full import of the discovery and establishment by Virchow of the principles of cellular pathology, that this constitutes the secure foundation upon which nearly two generations have built and future generations will continue to build the edifice of scientific medicine, I do not know what greater name there is in the whole history of medicine than that of Rudolf Virchow. How noble his character! With what amazing industry, versatility and keenness of intellect did he fruitfully cultivate the new fields which he had opened to research as well as other departments of science! With what devotion and beneficial results did he give his time and abundant knowledge to the service of the public and of our profession! We mourn the loss of a hero of medicine and of science, a benefactor of his race, and we rejoice in the rich fruitage of a long and well spent life.

The first place in experimental medicine today is occupied by the problems of immunity, and, in accordance with the trust of the Huxley lectureship, which provides that the lecture shall relate to "recent advances in science, and their bearing upon medicine and surgery," I have chosen for my theme "Recent Studies of Immunity, with Special Reference to their Bearing on Pathology." As it would be hopeless to attempt a complete review of this broad subject within the space of a single lecture, I shall dwell more particularly upon certain of its aspects, not always of necessity the most important ones, which I conceive to be less familiar to most physicians, or which have engaged my attention, although much I shall say is of course known to those who have followed the results of recent work in these lines of investigation.

Under "Studies of Immunity" I have included, as a matter of convenience, though not with strict accuracy, investigations which, although the direct outgrowth of those primarily directed toward a solution of the problems of immunity, have extended far beyond these bounds, and have revealed specific properties of cells and fluids in health and in disease of the broadest biological interest. We find illustrated here the familiar fact, nowhere more important to recognize than in medicine, that the sciences are interdependent, that discovery in one field sheds light in most diverse and often unexpected directions, and opens new paths to research. We shall see also exemplified the fructifying influence upon the advancement of knowledge of the discovery and application of new methods of investigation.

In endeavoring to follow in its intimate workings the contest of the living body with its invaders, the attention of investigators has naturally been drawn both to the action of the cells and to the properties of the fluids of the body in this struggle, to the latter sometimes without sufficient consideration of the dependence of the humors in their composition upon the cells. Each of these lines of study, whether followed separately or conjointly, has led to the discovery of important facts relating to the mechanism of immunity.

We owe to Metchnikoff and his pupils the most important observations concerning the direct participation of leucocytes and other cells in the processes of infection and the production of immunity. Whatever attitude one may take toward Metchnikoff's well known phagocytic theory of immunity, one must recognize the wealth of new facts which he has brought to light, and must admire the skill and fertility of resource with which for two decades he has defended this theory against severe assaults, and has done so, in my judgment, with a large measure of success. With wonderful ingenuity in his recent book on immunity he rescues the phagocytes and applies to a deeper insight into their activities results of his opponents' work.

The other line of research, in some respects more important, was opened by Nuttall in 1888, working in Flügge's laboratory, by his systematic study of the antibacterial properties of the body fluids, particularly of the blood serum. It is true that there were previous indications of the power of fresh blood to kill bacteria; indeed, if one wishes to trace this matter historically to its roots he must go back to John Hunter, who was quite familiar with the antiputrefactive power of fresh blood, although of course he knew nothing of bacteria. Hunter showed that putrefying fluid could be added in small quantity to fresh blood without setting up putrefaction; and in elaborating his favorite doctrine of the "living principle of the blood" he interested himself greatly in certain phenomena which, interpreted in the light of our present knowledge, are clear anticipations of some recent findings.

After Nuttall our knowledge of the bactericidal power of the blood serum was extended by Buchner and others; but the next advance of fundamental importance in this direction was Pfeiffer's discovery in 1894 of the quick extracellular disintegration and solution of cholera spirilla in the peritoneal cavity of immunized guinea pigs or in that of normal guinea pigs treated with immune serum, and of the presence in the immune serum of a specific substance concerned in the bacteriolytic process although by itself without bactericidal power.

In the meantime Behring had made his great discovery of antitoxic immunity and of the protective and curative value of antitoxic serum, and Ehrlich had done much to elucidate the nature of this form of immunity. It soon became apparent, however, that immunity from the great majority of bacterial infections does not depend in the main upon the antitoxic principle. The attention of bacteriologists, therefore, was drawn more and more to the so-called "Pfeiffer phenomenon," which was found to be of great general significance; and starting from this, and especially from the investigation of the analogous and much more readily studied solution of red corpuscles by foreign serum, there has followed in rapid succession up to the present time a series of new and most interesting discoveries and conceptions with which are connected many names, but most prominently those of Metchnikoff and Bordet, and of Ehrlich and Morgenroth.

Through these various studies of immunity we have become acquainted with an important physiological capacity of the healthy organism, the extent, and in most instances the existence, of which was unsuspected until quite recent years. This capacity is the power to produce substances specifically antagonistic to all sorts of foreign cells and cellular products and derivatives.

The substances capable of inducing this immunizing reaction appear to be mainly of an assimilable, albuminous nature, or at least intimately associated with such material, although it has been proved that certain nonalbuminous derivatives of proteids have the same power.[2] The mode of antagonism of the specific bodies formed in response to the reception within the living organism of substances capable of inducing the necessary reaction varies with the nature of these latter substances, and consists in such diverse manifestations as neutralization of poisons and of ferments, injury or destruction of cells, associated with characteristic morphological changes, cessation of motility of cells or their appendages, agglutination of cells, precipitation, and coagulation. In accordance with these different effects, the corresponding antagonistic bodies, or antibodies, as they are called, are classified as antitoxins, antienzymes, cytotoxins, agglutinins, precipitins and coagulins, and even against these bodies, with the exception of the antitoxins, antagonists have in turn been produced. All of these bodies are in varying, but usually high, degree specific with reference both to the nature and to the source of the material upon which they exert their characteristic effects.

The cytotoxins or cytolysins include not only the bacteriolysins and haemolysins, but also a great number of other cellular toxins present in the sera of animals which have received injections of cells from a different species. To every cellular group of an animal species there appears to correspond a specific cytotoxin. To designate these various cytotoxins such self-explanatory names as leucotoxin, spermotoxin, nephrotoxin, neurotoxin, thyreotoxin, syncytiotoxin are used. Their specificity extends not only to the nature of the cells, but also to the species of animal furnishing the cells used for their production.

One of the most important results of recent work is the separation of these specific antibodies into two groups, in one of which, represented by the antitoxins, the antagonists are single bodies; while in the other, represented by the cytolysins, the antagonistic effect requires the cooperation of two bodies. Of these two bodies the one which actually destroys the foreign cells, or induces other specific effect, is normally present in the cells or fluids of the organism, but it seems incapable of action without the intermediation of a body which is distinguished from it by greater resistance to heat, and which is produced by the immunizing reaction, although it may be also be normally present in smaller amount.[3] The two elements composing a cytolysin exist quite independently of each other, so that one may be present without the other, or be artificially removed without affecting the other. Of the multitude of names proposed for these cytolytic components those most commonly used for the body which is the specific product of immunization, although it may also exist normally, are intermediary body, immune body, amboceptor, sensitizer, fixative, preparative, desmon, and for the other body complement, alexin, cytase. It is this latter body which contains the atomic group described as toxophoric or zymophoric.

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[2] Specific precipitins have been produced by injection of crystalline and other so-called pure proteids. Obermayer and Pick produced immune bodies by the use of nonalbuminous products of tryptic digestion of certain albumins. Jacoby has shown that the specific body concerned in ricin immunization is nonalbuminous. A. Klein obtained entirely negative results with injections of starch, glycogen, glucose, gum arabic and gelatine.

Concerning the source, mode of action and constitution of the specific antagonistic-bodies we are very imperfectly informed. That they are of cellular origin seems certain, and Ehrlich with great ingenuity, on the basis of brilliant series of experiments, has advanced a hypothesis regarding them which, in my opinion, better than any other hitherto suggested, accords with the known facts, and in promoting discovery has already done the greatest service of which a working hypothesis is capable. Ehrlich has so recently and so fully in the Croonian lecture presented before English readers his hypothesis of the side chains or receptors and the basis for it, that I need only recall to your minds his conception that the toxins, cells and other substances which lead within the living body to the production of antitoxins, cytolysins and other antagonists have this capacity only through the possession of specific affinities, called haptophore groups, for corresponding haptophore groups belonging to side chains or receptors of certain cellular constituents of the body, and that in consequence of this appropriation of receptors, other of like nature are reproduced in excess of the needs of the cell, and these being shed into the lymph and blood, there constitute the antitoxins, intermediary bodies, agglutinins and other specific antagonists. The antitoxic receptor has only a single combining affinity, which is for the toxin, whereas the cast-off receptors constituting the intermediary bodies of cytolysins have at least two affinities (hence called amboceptors by Ehrlich), one of a more highly specialized nature being for the invading bacteria or other foreign cells, and the other for the complement.[4] The antibody enters quantitatively into definite chemical union with its affinitive substance. The essence of Ehrlich's theory concerning antitoxin is thus tersely expressed by Behring: "The same substance which, when incorporated in the cells of the living body, is the prerequisite and condition for an intoxication becomes the means of cure when it exists in the circulating blood." So of the twofold bactericidal and cytolytic agents we may say that the living body possesses substances which may protect it by destruction of invaders or may injure it by destruction of its own cells, according to the mates with which these substances are joined.

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[3] Metchnikoff believes that the complement or cytase, which in his opinion exists under normal conditions solely within cells, not free in the plasma, acts in natural immunity without the cooperation of an intermediary body or fixative, the latter being concerned only in acquired or artificial immunity. The evidence seems, however, to favor the view that in this regard the conditions are similar in both forms of immunity, the main difference being the presence of a much larger amount of the specific immune body in the latter.

An inquiry which naturally arises in this connection is: What is the physiological mechanism called into action in the processes resulting in the production of antitoxins, cytolysins and similar bodies? We have no reason to suppose that the animal body is endowed with properties specially designed to meet pathological emergencies. Its sole weapons of defense, often lamentably imperfect for morbid states, are adapted primarily to physiological uses.[5] To the foregoing inquiry Ehrlich answers that the mechanism concerned is one physiologically employed for the assimilation by the cells of food. The receptors are in cells, not for the purpose of linking poisons from the cells but to seize certain food stuffs, particularly the proteids, and the toxins and bacterial and other foreign cellular substances, if capable of inducing the immunizing reaction, chance to have the requisite combining affinities for the food receptors. It is interesting that Metchnikoff also, though from a different point of view, refers the mechanism of immunity to the physiological function of assimilation of food by the cells.

Inasmuch as, according to Ehrich's hypothesis, the specific antagonistic substances resulting from the injection of toxins and of foreign cells or derivatives of cells exist performed in cells of the normal body, there would appear to be no reason why any one of them might not occasionally be present normally free in the blood or other fluids. In fact, many of them—such as diphtheria and tetanus antitoxins, various antienzymes, bactericidal, haemolytic and other cellular toxins, agglutinins and a number of other bodies of this class, as well as their antibodies—have been found repeatedly, though of course in the case of many inconstantly and with marked differences between individuals and species, in the blood of healthy human beings or animals when their presence could not reasonably be attributed to a previous specific immunization. Of these normal antibodies the only one which is increased in amount by the process if immunization is that specifically related to the material used to bring about the reaction. As already stated, it is the intermediary body,[6] not the complement, which is generated in immunization against bacteria and other cells.

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[4] According to Ehrlich's latest conception, resulting from investigations to demonstrate the multiplicity of complements, an amboceptor has a single cytophilic affinity, and a number of complementophilic affinities differing in their avidity for various complements. He regards the agglutinins, precipitins and coagulins as uniceptors of more complex structure than the antitoxins, but Bail has recently brought evidence to show that agglutinins, like cytolysins, are composed of two elements. For the purposes of this lecture, it is not deemed necessary to enter into these or many other details of this complicated subject. For comprehensive and admirable critical reviews of recent theories of immunity and Ehrlich's hypothesis of the receptors, I would refer to Dr. Ritchie's papers in the Journal of Hygiene, Vol. II, No. 2, and succeeding numbers; and to Dr. Aschoff's paper in Zeitschrift f. allgem. Physiologie, Bd. I, Heft 3.

[5] W. H. Welch: Adaptation in Pathological Processes, Trans. Congress American Physicians and Surgeons, 1897, Vol. IV.

The foregoing statements, though of necessity condensed and incomplete, about the general characters of the specific antibodies will, I trust, help to a better understanding of what is to follow concerning the bearing of some of these discoveries on medical science and practice. I realize the difficulties which you must already have experienced, if unfamiliar with these new lines of research, in following a brief presentation of a subject in which not only are the facts so complex, and the ideas so novel, but the terminology so strange and burdened with such a multitude of confusing synonyms. While deploring the multiplication of unnecessary new terms, I should like to quote in this connection a wise remark of Huxley:[7]

> "If we find that the ascertainment of the order of nature is facilitated by using one terminology, or one set of symbols, rather than another, it is our clear duty to use the former; and no harm can accrue so long as we bear in mind that we are dealing merely with terms and symbols."

The most remarkable and characteristic attribute of these antibodies is the specificity of their relation to the substances which have led to their formation. Of some of them, such as diphtheria or tetanus antitoxin, this specificity is nearly absolute; of other, such as the precipitins, it is only relative. This property is the basis of new and most valuable methods for the identification of species and the determination of genetic relations—species not only of living things, but also of chemical substances and of disease.

The resemblances and the differences thus revealed are doubtless fundamentally of a physicochemical nature, but in many instances they transcend the powers of the microscope or of ordinary chemical tests to detect.

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[6] I use in this lecture the name "intermediary body" in preference to the more technical term "amboceptor," although Ehrlich applies the German equivalent—*Zwischenkörper*—only to normal as distinguished from immune amboceptors.

[7] Huxley: On the Physical Basis of Life, Collected Essays, Vol. I, p. 164, New York, 1893.

The results already attained by the method of serum diagnosis[8]—using this expression in its widest sense—are not only of interest and importance to the biologist, physiologist and chemist, but of great practical value to the bacteriologist and the physician. As this is not an aspect of my subject, broad and important as it is, upon the details of which I propose to dwell, it must suffice to present, by way of illustration, examples of the diagnostic application of different kinds of specific serums.

The only certain means of detecting toxins of the class of diphtheria or tetanus toxin, snake venom and certain vegetable poisons of the same category is their neutralization by the corresponding antitoxic serums. Occasion may arise where such detection is of practical and even medicolegal importance, as has been exemplified in India, where the criminal use of cobra venom is not unknown.

The application of serum diagnosis which is most familiar to physicians is the agglutinative test for typhoid fever. The principles of the agglutinative reaction were worked out in the laboratory of Professor Gruber in Vienna by himself and Durham, and were there first applied to the diagnosis of typhoid fever by Grünbaum, who was anticipated in his publication by Widal, who has made a thorough clinical study of the subject. The method is of great value, not only in the diagnosis of disease, but also in the identification of bacterial species and the recognition of relationships between species. Durham, to whom we owe important contributions to this subject, has given an ingenious hypothetical explanation of mutual agglutinative reactions, the main features of which are paralleled in Ehrlich and Morgenroth's doctrine, based upon experiments, relating to the multiplicity of cell receptors and of amboceptors concerned in haemolysis.[9]

We have found the agglutinative reaction an indispensable aid in the study of the series of cases of paratyphoid fever which have come under observation in Dr. Osler's wards at The Johns Hopkins Hospital, and which otherwise it would have been scarcely possible to have separated from typhoid fever.[10] The occurrence of paratyphoid fever as a distinct disease affords an explanation of a certain proportion of the failures of the serum from supposed typhoid fever patients to clump typhoid bacilli. Not less valuable is the serum test in the diagnosis of *Bacillus dysenteriae* Shiga and of the diseases caused by it. This microorganism has been shown by Flexner and his pupils, Vedder and Duval, to be the cause of our acute dysenteries, and recently in Baltimore Duval and Bassett, working with the aid of the Rockefeller Institute for Medical Research at the Thomas Wilson Sanitarium for Children, have discovered that this same bacillus is in all probability the specific agent of infection in at least many of the summer diarrhoeas of infants.

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[8] The general procedure followed in the production of specific serums is the injection into a suitable animal at intervals of time repeated doses of toxins, bacteria, foreign cells, or other material against which the antibody is desired. For example, if a specific precipitating or a haemolytic serum for human blood is wanted, an animal, say a rabbit, is injected subcutaneously or intraperitoneally at intervals of three or more days with five or six doses of human serum or human red blood corpuscles. At the end of this time the rabbit's serum has acquired the property of precipitating human serum in strong dilutions, or of dissolving human red blood corpuscles, if these were used for the injection. Within limits the less closely related the two species of animals the more powerful is the antagonistic effect of the specific serum. This is true especially in the case of cytotoxic serums.

[9] Durham: Journal of Experimental Medicine, January 15, 1901, Vol. V, p. 353. Ehrlich and Morgenroth: Berl. klin. Woch., May 27, 1901, p. 570.

Bacteriolytic serums have been used by Pfeiffer in the differentiation of cholera and allied spirilla, but few other bacteria present equally well the Pfeiffer reaction, which is not nearly so useful or handy a means of identification as the Gruber-Durham reaction.

Of other cytolytic serums the haemolysins have been by far the most carefully studied. One of the most interesting results of this study has been the determination by precise quantitative methods of resemblances and of differences between red blood corpuscles which in no other way could be distinguished. These resemblances and differences relate to the red corpuscles not only of different species of animals, but also to those of individuals of the same species. Although we constantly assume the existence of cellular differences between individuals and between species, these are for the most part of so subtile a nature as to elude our methods of observation. The exact demonstration of such differences by the use of cytolytic serums is therefore of especial interest. My assistant, Dr. H. T. Marshall, in an unpublished research, conducted under the direction of Professor Ehrlich and Dr. Morgenroth, upon the receptors of the red blood corpuscles of man and of two species of monkey, found that while man and the monkeys each have receptors not shared by the other, they also have a large number of receptors in common.

This result is in harmony with Nuttall's interesting observations on a much more extended scale regarding phylogenetic relationships between animal species, as shown by the reaction of their blood with the specific precipitins discovered by Tchistowitch and Bordet, and introduced into practical medicine by Wassermann. This biological test to determine the source of blood, when used with proper precautions, far surpasses in accuracy all other methods for this end. While it would lead too far from my purpose to follow this subject farther, I cannot in this connection forbear at least mentioning one of the earliest and most suggestive papers on this class of antibodies—that "On Immunity Against Proteids," by Walter Myers, who gave up his life in the cause of science and of humanity, and whose early death is so great a loss to English medical science.

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[10] See papers on paratyphoid fever, by Johnston, Hewlett and Longcope in American Journal of Medical Sciences, August, 1902.

I shall ask your attention now to some considerations concerning the bearing of recent studies of immunity on the nature and action of toxins. This subject is, of course, of the greatest pathological as well as bacteriological importance, and I believe a closer cooperation than now exists between bacteriologists and pathologists in its study would further the surer and more rapid advancement of our knowledge about it. One misses only too often in purely bacteriological papers on this subject exact knowledge and descriptions of pathological conditions, and, on the other hand, pathologists often fail to utilize pertinent facts and ideas which are familiar to bacteriologists.

The discovery by Roux and Yersin of the diphtheria toxin, the studies by Behring and Kitasato of tetanus toxin leading up to Behring's epochal discovery of antitoxin, and the later investigations of Ehrlich on the constitution of diphtheria toxin and the origin and mode of action of antitoxin are the great events in the most brilliant and securely founded chapter of modern studies of immunity. Through these researches we became acquainted with a class of poisons secreted by certain bacteria, and present in solution in culture fluids. The evidence is conclusive that these soluble toxins enter, as assimilable substances, into direct combination with constituents of the body cells for which they have an affinity, and only thereby are enabled to bring about immunity or to exert toxic effects. As shown by the modifications of toxoids, the toxic property may be destroyed without loss of the combining power, and without removal of the immunizing power. According to Ehrlich's helpful conception, based on a large amount of experimental evidence, and now very generally accepted, the combining power of the toxin molecule resides in a group of atoms, designated as the haptophore group, with affinity for the corresponding haptophore groups of the side chains or receptors of cellular constituents, and the toxic power pertains to another and less stable atom complex in the molecule.

By means of these facts, and legitimate deductions from them, we are enabled to explain in a satisfactory way susceptibility to poisoning by these soluble toxins, their selective action upon the cells of the body, and their quick disappearance after injection into the circulating blood. In one infectious disease and in one only, to wit tetanus, are we able to explain the clinical and pathological phenomena in minute detail on the basis of our knowledge of the causative microorganism and its poisonous products. The nearest approach to this instance is diphtheria, but here we have not yet been able to follow the trail of the toxins within the body so perfectly, and, as Flexner and I have shown, in addition to the soluble toxins there is an intracellular poison concerned in the production of the false membrane. Interesting investigations, which have greatly helped to elucidate the nature of these toxins, have been made on various similar vegetable and animal poisons, such as ricin and abrin from the former source and the venom of snakes, spiders and other poisonous animals.

The high hopes which were raised by the discovery of the soluble bacterial toxins that at last the way was opened for us to penetrate into the mysteries of the mode of action of pathogenic bacteria were soon doomed to disappointment, for similar powerful toxins, though diligently sought, could not be detected in the cultures of most other bacteria, and these among the most important ones, such as the tubercle bacillus, the typhoid bacillus, the cholera spirillum, the pneumococcus, the pyogenic micrococci. This disappointment was all the more acute because there was and is every ground for confidence that whenever we have in our possession a powerful toxin of this class, a strong protective antitoxic serum can readily be obtained.

Notwithstanding these negative results, the belief was not only abandoned that bacteria harm the body mainly by poisoning, for it rests upon strong clinical and pathological evidence, as well as upon the study of the distribution of bacteria in the infected body. The search for poisons was turned from the fluid part of cultures to the bacteria themselves, and thus Pfeiffer succeeded in demonstrating as an integral constituent of the bodies of cholera spirilla toxic substances, which are liberated only when the bacteria degenerate or die. Intracellular poisons, which indeed previously, though of a different nature, had been extracted from bacteria by Buchner and by Koch, were subsequently found within typhoid bacilli and a number of other pathogenic bacteria.

It is of more than purely bacteriological interest to recognize the distinction between the small group of pathogenic bacteria, represented by the bacilli of tetanus, of diphtheria, and of botulism, characterized by the secretion of powerful soluble toxins, and the much larger group, containing most of the other pathogenic bacteria, which do not secrete similar toxins, for it is only the former which give rise to the production of antitoxic serum of marked protective and curative power.

The form of immunity resulting from injections or natural infections with the second class of bacteria belongs mainly to the bacteriolytic type, in which the complete antibody is not a single substance, like antitoxin, but is composed of two distinct elements, intermediary body and complement, of which only the former is produced or increased in the process of immunization. The bacteriolytic serums are also under suitable, but not readily controlled conditions, protective and curative, but owing, it would seem, mainly to the duplex nature of the antibody their successful therapeutic application meets difficulties which have not yet been overcome. The great practical problem of bacteriology today is to make available to medical practice the bacteriolytic serums such as antityphoid, antipneumococcus, antistreptococcus, antiplague, antidysentery serums. Such work as that of Marmorek, of Wassermann, of Neisser and Wechsberg, of Ainley Walker, upon the production, the properties, the conditions underlying the action of these serums is, therefore, highly important.

Our knowledge of the constitution and action of the intracellular bacterial poisons is most incomplete and at present cannot be applied in any very definite and satisfactory way to the explanation of the morbid phenomena of infectious diseases. Such investigations as those undertaken by Macfayden and Rowland at the Jenner Institute of Preventive Medicine upon the expressed juices of bacterial cells promise to shed light upon this subject and in general upon the vital processes of bacteria. Of great value also are the recent researches of Vaughan upon intracellular bacterial poisons.

I find it difficult to reconcile myself to the doctrine that bacteria, such as the typhoid bacillus, the pneumococcus, and others of the class now under consideration, do their chief injury to the body, not while they are lively and vigorous, but after they become corpses and in consequence set free their protoplasmic poisons. Still this latter conception is the basis if a coherent hypothesis of infection, elaborated most fully recently by Radziewsky,[11] which rests upon a considerable amount of accurate observation and interesting experimental work. There can be no doubt that in the course of many infections there goes on an enormous destruction of the bacteria concerned so that the numbers of those indicated at any given time by microscopical examination and by cultures may represent only an insignificant fraction of the total progeny of the first invaders. I have been much interested in this phenomenon, since I became familiar with it over twelve years ago[12] in pneumococcus infections through the employment of a method which revealed in the exudates degenerating and dead pneumococci and their empty capsules in numbers often far exceeding the intact organisms; indeed, in some cases so many that they formed a large part of the exudate.

While all due weight should be given to such facts as these, the objections to the acceptance of the hypothesis just mentioned as affording a complete explanation of the toxic phenomena of this class of infections are so obvious that naturally efforts have been made to learn whether bacteria which produce no strong soluble toxins in our ordinary culture media may not do so on other media of special composition or in a demonstrable way within the living body. Work in these two directions has not been altogether barren, as shown by results of experiments made by Hueppe, Cartwright Wood, Marmorek, and others along the former lines, and by Metchnikoff, Roux and Salimbeni along the latter, but it cannot be said that these experiments have led to any generally accepted solution of the main difficulties. Some are therefore inclined to lay the chief emphasis upon disordered cellular metabolism, but this is only a restatement of the question. Everybody recognizes abnormal metabolism as an essential condition in infections. The very point needing explanation is how the bacteria derange metabolism.

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[11] Radziewsky: Zeitschrift für Hygiene, 1900, XXXIV, p. 185, and 1901, XXXVII, p. 1.

[12] Welch: Bulletin of The Johns Hopkins Hospital, July, 1890, and December, 1892. Michaelis: Berl. klin. Woch., 1902, No. 20, has recently reported the same findings.

I wish here to advance a hypothesis which seems competent to explain the source, the mode of production and the nature of certain bacterial toxins. It would appear to be a natural inference from the receptor theory of Ehrlich and the recent work on cytotoxins. The following considerations will, I hope, make clear the essential points.

As I have already stated, we know that the injection of foreign cells, such as pathogenic bacteria, red blood corpuscles, spermatozoa, epithelium, into the tissues of a living animal leads to the formation of poisons, called cytotoxins, acting specifically upon these cells; that the substances which stimulate the cells of the host to produce one constituent of this class of toxins consist of certain atom complexes derived from the injected cells; that certain cells of the host thus stimulated generate and discharge one component of the toxin, called the intermediary body, which, although by itself not poisonous, becomes the medium of intoxication through union, on the one hand, with a preexistent toxophore substance, called the complement, and, on the other hand, with the foreign cell which started the reaction.

Such is the response on the part of the host to the entrance of the foreign cells; but how about a possible response of a like nature on the part of the invading cells toward the host resulting in the production of special cytotoxins, of analogous constitution, injurious to the host? This latter response, being of a vital nature, can take place only when the invading cells are living, as in the case of bacteria and other parasites.

I see no reason why suitable substances derived from the host may not stimulate parasitic organisms, through a physiological mechanism similar to that operative in the development of cytolytic immunity, to the production of intermediary bodies which, if provided with the requisite affinities, have the power to link complements to cellular constituents of the host, and thereby to poison the latter. Expressed in terms of Ehrlich's side-chain theory, certain substances of the host of the cellular origin, assimilable by the parasites through the possession of haptophore groups with the proper affinities, become anchored to receptors of the parasitic cell, which, if not too much damaged, is thereby stimulated to the over-production of like receptors; these excessive receptors of the parasite, if cast off into the fluids or the cells of the host, there constitute intermediary bodies or amboceptors with special affinities for those cellular constituents or derivatives of the host which led to their production, and for others which possess in whole or in part identical receptors. Provided the host is supplied also with the appropriate complements, there result cytotoxins with special affinities for certain definite cells or substances of cellular origin in the host. The contribution of the parasitic cells to these cytotoxins is the amboceptors. Either the parasite or the host may provide the complements.[13]

It may perhaps aid in grasping the ideas here presented to imagine the bacterium, in the capacity of the host, as a structure so large that one could inject into it animal cells. Provided the proper receptor apparatus is present, the resulting reaction on the part of the bacterium, as described, would be a process of immunization against the animal cells through the formation of specific cellulicidal substances. In reality it is only certain atomic complexes of cells which are concerned in this immunizing reaction, and in comparison with these even the smallest bacterium is a gigantic object.

Looked at from the point of view of the bacterium as well as from that of the animal host, according to the hypothesis advanced the struggle between the bacteria and the body cells in infections may be conceived as an immunizing contest in which each participant is stimulated by its opponent to the production of cytotoxins hostile to the other, and thereby endeavors to make itself immune against its antagonists. These mutually antagonistic cytotoxins are capable of injuring the parasitic cells on the one hand or the body cells on the other, only when escaping combination outside of them they are anchored to the receptors of the cells to which their respective affinities are adjusted. This combination with the cells, if it does not result in too great injury to them, is the condition for further production of the cytotoxic intermediary bodies through over-production and discharge of receptors.[14] The important factors determining the issue of the contest are the qualities, the relative proportions and the distribution of the bacterial and the host's cytotoxins.

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[13] We may thus speak of somatogenic cytotoxins resulting from the action of bacterial stimuli on cells of the host, and of bacteriogenic cytotoxins from somatogenic stimuli, also of somatogenic and bacteriogenic complements.

[14] It will be observed that these discharged receptors may be regarded as the equivalents of anti-immune bodies. E. W. Ainley Walker, in an interesting and suggestive paper on Immunization against Immune Serum (Journal of Pathology and Bacteriology, March, 1902), shows experimentally that bacteria growing in immune serums produce anti-immune bodies, and thereby become more

The hypothesis thus outlined can be tested experimentally, but I regret that it has shaped itself in my mind so recently that I have not yet been able to make the desired experiments, which are, however, now started in my laboratory. Since my arrival here I am informed that these experiments have already furnished facts in its support, which will be published later.

Inasmuch as at least one component, and it may be both components, of the assumed bacterial cytotoxins preexist in the bacterial cells, it should be possible to demonstrate some of them in artificial cultures of bacteria, where they would be found especially as integral parts of the cells, unless extracted from the bodies of degenerating or dead bacteria. This corresponds with what is known concerning the situation of the poisons of the cholera spirillum, the typhoid bacillus and other bacteria characterized by the lack of strong soluble toxins. But the quantitative and other relations between these cultural cytotoxins and those produced in the manner described by the same bacteria during processes of infection are comparable to those between the normal antibodies and the immune antibodies. These relations

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virulent. He concludes that " the basis of bacterial virulence and of chemiotactic influence is identical, and constitutes that atom group which causes the production of the immune body." My hypothesis includes the conceptions supported by Walker, and also much more. According to the hypothesis, certain bacterial antibodies are capable not only of neutralizing immune bodies of the host, but with the aid of complements also of poisoning the cells of the host. It is not difficult to imagine various conditions in which the antibodies of bacterial origin may escape neutralization before entering into union with the host's cells. The substances which stimulate bacteria to produce these antibodies need not necessarily be toxic to them; in fact, toxicity, such as that of strong bactericides of cellular origin, would hinder their production. The essential things are that the stimulating substances have the requisite combining groups for bacterial receptors, and that the cast-off receptors be complemented within the body of the host. Each of the various bacteriogenic cytotoxins probably contains a multitude of partial amboceptors, with varying cytophilic and complementophilic affinities, in accordance with the views of Ehrlich and Morgenroth. It is self-evident that through a mechanism similar to that described parasites within the infected body may be stimulated by atom groups derived from the host to the production also of antibodies other than cytotoxins, such as various agglutinins, precipitins, antienzymes, and perhaps of uniceptors of the nature of secreted soluble toxins, or of enzymes, all adjusted against the host. Questions relating to the source and nature of the complements, particularly of intracellular complements, and also to anticomplements, are manifestly of importance in relation to the hypothesis, but it would complicate the subject too much to discuss these and other matters here, where my purpose is merely to outline the essential features of this new theory of infection with reference to the particular points under consideration.

would explain the familiar fact that cultures of bacteria of the class under consideration constitute in general only a partial and meager index of the toxic capacities of the same bacteria in the infected body. That cytolysins may, however, be present normally in large amount is illustrated by the haemolysins of eel's serum and of snake venom.

In this theory, degenerated and dead bacteria, while recognized as a source of poisoning in infections, are not assigned an exclusive rôle in this regard. Living bacteria in the infected body, where they are under nutritive conditions not paralleled in artificial cultures, actively produce and secrete receptors which may become the means of intoxication of the body cells. From what has been said, we can comprehend how these diverse free receptors may enter into the formation of cytotoxins of the most varied and specific characters, such as erythrotoxins, leukotoxins, neurotoxins, nephrotoxins, spermotoxins, hepatotoxins, etc. Very probably in many instances these toxins are represented by so few receptors in bacterial cells in ordinary cultures that it would be hopeless to search for them there, although we may have convincing experimental and pathological evidence that within the animal body the same bacteria produce them abundantly under the stimulus of appropriate substances derived from cells of the host.

The methods hitherto employed for the study of bacterial poisons have not generally been calculated to reveal the presence of toxins with the characters indicated, even if such existed in the cultures. Recently, however, a beginning has been made in this direction, and we have already become acquainted with certain toxins of an interesting nature, to which I desire to direct your attention.

Intrinsically and in their general bearing upon the subject before us, the recent investigations of Flexner and Noguchi upon the constitution of the toxins in snake venom are of special importance. It was in snake venom that Weir Mitchell and Reichert first demonstrated the existence of that class of poisons often called, although with doubtful propriety, toxic albumins. Investigations of snake toxins are of peculiar interest for many reasons, not the least of which is their resemblance to bacterial toxins. The demonstration by Sewall of the possibility of active immunization from venom, and the further studies by Calmette and by Fraser of this phenomenon, and especially of the protective and curative properties of antivenin are well known.

Until recently it has been generally held that the venom toxins resemble in molecular structure the diphtheria and the tetanus toxins in being single bodies with a combining or haptophore group and a toxophore group of atoms. The researches of Flexner and Noguchi, now in progress, of which only the first part has been published,[15] necessitate a quite different conception of the nature and manner of action of venom toxins from that previously entertained. I have followed with great interest the work of Professor Flexner on toxins, begun several years ago in my laboratory when he was my assistant and associate, and since continued along new lines in his laboratory at the University of Pennsylvania, and I wish to acknowledge his generosity in permitting me to use in this lecture certain unpublished results of his and Noguchi's investigations.

These investigations have shown that the toxic action of venom upon red blood corpuscles, leucocytes, nerve cells and other cells is like that of the duplex cytotoxins already described—that is, it depends upon the combination of intermediary bodies contained in the venom, on the one hand with the animal cells for which these bodies respectively have affinities, and on the other hand with corresponding complements contained, not in the venom, but in the cells or fluids of the animal acted on. For example, it is well known that the addition of venoms to fresh blood brings about the quick destruction and solution of the red corpuscles. If, however, certain venoms be added to red corpuscles which have been thoroughly washed with isotonic salt solution so as to remove all the complement, the corpuscles are agglutinated but not dissolved, although it can be shown that substances from the venom (intermediary bodies) have entered into combination with the corpuscles. If now a little fresh serum which contains the complement, and by itself may be an excellent preservative of normal corpuscles, be added to these venomized corpuscles, they are promptly dissolved.

Preston Kyes, working in Professor Ehrlich's laboratory, in an investigation just published[16] on the mode of action of cobra venom, confirms the conclusion of Flexner and Noguchi concerning the amboceptor nature of cobra venom, and adds much that is new and important to our knowledge of this subject. He finds that the washed blood corpuscles of certain animals are directly dissolved by cobra venom, while those of other animal species require the subsequent addition of complements or adjuvants to bring them under the influence of the venom. But even in the former case a complementary body is essential to the reaction, this, however, being not a serum complement, but an endocomplement contained within the red corpuscles. Of great significance is the demonstration by Kyes of still a third substance, namely lecithin, which is capable through combination with the venom intermediary body of completing the haemolytic potency of venom.[17] The discovery for the first time of a definite, crystallizable substance with the power of uniting, like a complement, with an intermediary body, and thus completing the formation of a cytotoxin, is evidently of fundamental importance. The suggestion by Ehrlich and Kyes that possibly the cholin group is the toxophore group of lecithin is particularly interesting in the light of F. W. Mott's valuable studies of chemical processes concerned in degenerations of the nervous system.

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[15] Flexner and Noguchi: Snake Venom in Relation to Haemolysis, Bacteriolysis and Toxicity, Journal of Experimental Medicine. March 17, 1902, Vol. VI, p. 277.

[16] Preston Kyes: Ueber die Wirkungsweise des Cobragiftes, Berl. klin. Woch., 1902, Nos. 38 and 39. I am greatly indebted to my friend, Professor Ehrlich, and to my former pupil, Dr. Kyes, for putting me in possession of the main results of these interesting experiments before the date of their publication.

The researches of Flexner and Noguchi and of Kyes, therefore, have taught us that in poisoning by venom the bodies of human beings and of animals contain in the form of complements, or alexins[18] as they are also called, the substances which are most directly concerned in the act of poisoning. The venom serves merely to bring into the necessary relation with constituents of the body cells poisons we already harbor or may generate, but which are harmless without the intervention of intermediary bodies. These poisons within us are powerful weapons, which when seized by hostile hands may be turned with deadly effect against our own cells, but which are also our main defence against parasitic invaders. We see here as everywhere that nature is neither kind nor cruel, but simply obedient to law.

Flexner and Noguchi have demonstrated experimentally that, like the haemolytic, so also the leukotoxic, the neurotoxic, and other cytotoxic properties of venom depend upon combinations of venom intermediary bodies with complements contained in the cells poisoned by venom or in the fluids bathing these cells. Particularly striking are their experiments showing *in vitro* and under the microscope the cytolytic action of cobra venom upon certain large molluscan nerve cells in the fresh state. The complement essential to this reaction is contained within the nerve cells. In previous experiments of Flexner and Noguchi there had been indications that a special class of intracellular complements are concerned in some of the toxic effects of venom upon cells. The positive demonstration by Preston Kyes of a special class of intracellular complements or endocomplements is unquestionably of great pathological interest, and seems destined to play an important part in the explanation of many morbid conditions in connection both with endogenic and with exogenic intoxications, probably also in such phenomena as self-digestion or autolysis.

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[17] The objections made by Calmette (Compt. Rend. Acad. des Sc., 1902, T. CXXXIV, No. 24) to Flexner and Noguchi's interpretation of their experiments as to the amboceptor nature of venom have been completely overthrown by the experiments of Preston Kyes.

[18] There is some objection to the use of the term "alexin" as a synonym for "complement" as the former was applied originally by Buchner to substances which we now know to be combinations of complements with intermediary bodies.

Snake venom is a rich mine of diverse toxins, and, on account of its pathological importance, I must mention one of the cytotoxins discovered there by Flexner and Noguchi, as it may be that a similar toxin is produced by certain bacteria, and under still other conditions. As is well known, one of the most striking lesions resulting from poisoning by certain venoms is the occurrence of abundant haemorrhages in various tissues of the body. This effect has been generally attributed to the direct action of venom on the red corpuscles and on the coagulability of the blood, but the experiments of Flexner and Noguchi indicate that these haemorrhages are due to the presence in venom of a cytotoxin which has the power of dissolving endothelial cells—in other words, an endotheliolysin. Dr. Flexner suggests the name "haemorrhagin" for this special toxin which causes extravasations of blood through its direct solvent action upon capillary endothelium, an effect which is readily demonstrated under the microscope. It is hardly necessary for me to stop to emphasize the clinical and pathological importance of the discovery of an endotheliotoxin, a kind of poison which may prove to be of special significance in the interesting group of haemorrhagic infections, and perhaps also in purpura and kindred affections.

The foregoing newly discovered facts, which I have sketched only in bare outline, illustrate in a striking way the fruitfulness of methods and conceptions which we owe to recent studies of immunity. The results of these investigations, however, are significant beyond the mere facts disclosed, important as these are. They have for the first time revealed in normal toxic secretions, readily introduced under conditions of nature into the tissues of man and animals, cellular poisons akin to the complex haemolysins, neurotoxins, and other cytotoxins of immune and some normal serums, which have aroused so much interest and experimental study during the past four years. The most noticeable difference between the venom cytotoxins and those hitherto observed in immune serum is the far greater resistance to heat of the intermediary bodies of the former; but we are already acquainted with considerable variations in the sensitiveness to heat both of different intermediary bodies and of complements. That snake venom should contain only one-half of the complete poison, the other and the really destructive half being widely distributed in the blood and cells of man and of animals, is an instance of a curious kind of adaptation, of interest from evolutionary, as well as from other points of view.

In consideration of the often emphasized analogies between venom toxins and bacterial toxins, these facts render it highly desirable to make a systematic search of bacterial cultures by proper methods and under suitable conditions for complex cytotoxins. At present substances of this nature are not known to exist in our cultures. There have been discovered, however, within the past three or four years certain bacterial toxins which have a curious resemblance in some of their properties to the complex antibodies of blood, although so far as they have been carefully studied, they appear to have the simpler constitution of the soluble toxins, like those of diphtheria and of tetanus. I refer to the bacterial haemolysins, leucolysins, haemagglutinins, precipitins and coagulins. There is no reason to suppose that this list exhausts the number of those actually present, for it is evident that it includes chiefly bodies readily demonstrable in test tube experiments. It would be surprising if cytotoxins which act specifically upon red and white corpuscles were the only ones of this class produced by bacteria; in fact, we have every reason from pathological observations to believe the contrary.

It has become evident that more refined methods than mere observation of the coarse effects of injecting into animals the filtrates or the killed bacteria of our cultures are required for the detection of the subtler and more specific cellular poisons. Instances are rapidly increasing in which by improved methods cultures of bacterial species once believed to be practically devoid of toxicity are found after all not to be so poor in toxins, even of the soluble variety. One of the earliest and most instructive illustrations of this is the discovery by Van De Velde of a leucocyte-destroying poison, named leucocidin, in exudates caused by infection with *Staphylococcus aureus,* and also in filtrates of staphylococcus cultures, which had been previously regarded as almost entirely free of toxic power.

More widely distributed in cultures of different species of bacteria are the haemolysins, of which the first example, discovered in 1898 by Ehrlich in cultures of the tetanus bacillus, was carefully studied by Madsen the following year, and which have since been investigated by Kraus with Clairmont and with Ludwig, Bulloch and Hunter, Neisser and Wechsberg, Todd, Besredka and others. The list of bacterial species known to produce in cultures substances of this nature capable of dissolving red blood corpuscles is already a long one, and includes the bacilli of tetanus, of green pus, of typhoid fever, of acute dysentery, of diphtheria, of plague, the pyogenic staphylococci and streptococci, the pneumococcus, and many other bacteria. Nuttall and I noted in our first descriptions of *Bacillus aerogenes capsulatus* over ten years ago its capacity of laking blood, so that I was not surprised to find recently that a haemolysin can be demonstrated in cultures of this organism. The blood-destroying property appears to stand in no definite relation to virulence, nor is it limited to pathogenic bacteria. It pertains also to many putrefactive bacteria. The strongest bacterial haemolysin hitherto observed was found by Todd in cultures of *Bacillus megatherium,* which is a widely distributed saprophyte.

As already stated, none of these bodies has been shown to belong to the class of complex haemolysins in blood, which have been far more exhaustively investigated than any other of the specific antibodies. Doubtless there is at present among bacteriologists too great a tendency to attribute to the less carefully studied antibodies characters which have been worked out in detail only for the haemolysins of immune serum. It would lead too far to attempt here a discussion of the special characters of the various bacterial haemolysins, which present in different specimens curious and at present unexplained divergences as regards resistance to heat and several other properties. It must suffice to indicate briefly what is known of the pathological importance of this interesting group of bacterial toxins.

In view of the abundant clinical and pathological evidence of extensive destruction of red corpuscles in the course of many infectious diseases, it is certainly significant to find that many bacteria are endowed with a specific haemolytic power. The question is how far we are justified in applying to the actual conditions of infection the existing experimental data upon this subject. Assuredly here, as everywhere, results of test tube experiments, helpful in suggestion as they may be, should not be utilized without further evidence to explain morbid phenomena within the infected human or animal body. While much more work upon this subject is needed before our information will be exact or complete, the observations and experiments of Besredka,[19] Kraus and Ludwig,[20] and others have already demonstrated that bacteria may exert their blood-destroying power within the living body. This haemolytic capacity of microorganisms affords an explanation, although certainly not the only one, of the secondary anaemias which are such a marked feature of many infectious diseases, as streptococcic and other septicaemias, pneumonia, typhoid fever, scarlatina, and others. The haemoglobinuria which is a recognized although rare complication of various infectious diseases may be referable to intoxication with unusually powerful bacterial haemolysins, or to an exceptional lack of resistance of red corpuscles.

Haemoglobin, however, is not necessarily present in solution in the blood plasma, for the destruction of the damaged red corpuscles may take place within the large phagocytes of the spleen and the haemolymph glands, as is well known to occur on an extensive scale in typhoid fever and some other infections. A familiar example of the action of bacterial haemolysins is the post mortem reddening of the inner lining of the heart and blood vessels, an effect which may be due to putrefactive bacteria or may appear very soon after death, especially from septicaemia caused by *Streptococcus pyogenes,* which, as has been shown, may take the blood during life.

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[19] Besredka: Annales de l' Institut Pasteur, 1901, XV, p. 880.

[20] Kraus and Ludwig: Wien. klin. Woch., 1902, p. 382.

The fact that certain common saprophytic bacteria may produce energetic haemolysins, as pointed out by Kraus and Clairmont and by Todd, has a possible bearing upon the etiology of certain obscure anaemias not of infectious origin, particularly upon the interesting observations and the theory of William Hunter concerning their causation by absorption of toxins from the alimentary tract. Todd found cultures of *Bacillus megatherium* so strongly haemolytic that the intravenous injection of 1 c. c. of the filtrate into guinea pigs was followed by haemoglobinuria, 10 c. c. being fatal. Human red corpuscles are sensitive to this haemolysin.

Normal human and other blood serums contain in varying amounts antihaemolysins, which protect the red corpuscles from the action of some of the bacterial haemolytic agents. Specific antihaemolysins are readily produced by immunizing injections of bacterial haemolysins, and are generated also in the course of infections. Lang suggests that the augmentation of the osmotic resistance of the erythrocytes which has been noted in some infectious diseases, as well as in icterus and some other morbid conditions, may be a reactive phenomenon caused by the presence of haemolytic toxins.

Intimately associated with the haemolysins in cultures are the bacterial haemagglutinins,[21] substances which have the power to clump red blood corpuscles. Among unicellular organisms both the capacity to produce agglutinins and the aptitude for agglutination seem to be very widely distributed. The bacterial haemagglutinins, in analogy with the bacterial haemolysins, are apparently of simpler constitution than the serum agglutinins, being destroyed at 58° C., whereas the latter are not injured by temperatures under 70° C. In order to demonstrate in cultures the haemagglutinins it is generally necessary to eliminate in some way the action of the associated haemolysins, which can be done by using small quantities of the culture fluid or by keeping the mixture of fluid and red corpuscles at zero temperature.

I know of no observation directly demonstrative of the action of bacterial haemagglutinins within the living body in infections, but this subject is of such recent knowledge that it has been as yet scarcely investigated. Certainly there are morbid conditions which seem highly indicative of the operation of substances agglutinative of red corpuscles. Probably every one with large experience in the examination of fresh blood in disease has noticed that sometimes red corpuscles, examined immediately after with-

[21] Kraus and Ludwig: Wien. klin. Woch., 1902, p. 120.

drawal of the blood, have a peculiar tendency to form clumps which cannot readily be broken up. This phenomenon, which is certainly suggestive of the action of an agglutinating agent, I have observed especially in some cases of septic infections and of cirrhosis of the liver.

Furthermore, I would emphasize the support given by the recognition of haemagglutinins to views advocated many years ago by Hueter and by Klebs concerning the occurrence of thrombi composed of coalesced red blood corpuscles. Such thrombi I believe to be not uncommon in typhoid fever and other infections, especially in small blood vessels. I have elsewhere called attention to the evidence in favor of the interpretation of many of the hyaline thrombi as derived from agglutinated red corpuscles.

It can scarcely be doubted that substances agglutinative of white blood corpuscles are also produced by certain bacteria, and that these are concerned in the clumping of pus cells and of leucocytes within the living body, but it would not be profitable to discuss this matter without more exact information than we now possess.

In this connection I may say that not only the discovery of the bacterial haemagglutinins, but also that of the haemolysins and the leucolysins, is likely to shed new light upon certain aspects of the difficult subject of thrombosis. The red corpuscles undergo various morphological changes under the influence of different bacterial haemolysins acting with varying intensity. Distortions of shape, throwing out of projections, and detachment of colorless particles resembling platelets, can sometimes be seen. These observations are of special interest with reference to the doctrine, already strongly supported, that platelet thrombi originate from disintegrated red corpuscles. Levaditi, and Neisser and Wechsberg, have described, as the result of intravenous injections of *Staphylococcus aureus,* areas of coagulative necrosis in the rabbit's kidney, which they attribute to thrombi composed by disintegrated leucocytes caused by the staphylococcus leucocidin, to which I have already referred.

I have dwelt at some length, although of necessity incompletely, upon the bacterial haemolysins, leucocidins and haemagglutinins, because we are better informed about these agents than concerning other members of this recently recognized class of bacterial toxins. I have already expressed the opinion that similar poisons acting specifically upon other cells of the body are produced by bacteria; indeed neurotoxins and nephrotoxins of this type have been reported. The difficulties in the way of direct proof of the existence of these other bacterial cytotoxins are greater than in the case of those acting upon the red and the white blood corpuscles, but doubtless they can be overcome. Of course we have evidence of the action of bacterial poisons upon various body cells, but this is not enough. At present we can apply only in a vague and unsatisfactory way to the explanation of pathological processes most of the knowledge of this kind which we possess. What is urgently needed is a separation of these poisons and a determination of their source, constitution, mode of action and degree of specificity along such lines as have been followed so fruitfully in the investigation of the soluble diphtheria and tetanus toxins, those other toxins of bacteria and of venom already considered, and the cytotoxins of normal and of immune serum. The path leading apparently in the right direction has already been opened, and, if I mistake not, its further pursuit is most promising of valuable results in the near future.

Consider by way of illustration how helpless we now are in our efforts to explain the characteristic lesions of typhoid fever on the basis of our knowledge of the properties of the typhoid bacillus. That these lesions are referable to the action of toxins cannot, I think, be seriously questioned. Especially from the investigations of Mallory, we know that the most characteristic histological changes of this disease consist in the proliferation of the reticular or so-called endothelial cells of the lymphatic tissue of the intestine and the mesenteric glands and of similar cells in the splenic pulp, and in the assumption by these proliferated cells of remarkable phagocytic activities towards the lymphocytes in the former situations and towards the red corpuscles in the spleen. Mallory believes that these changes are best interpreted by supposing that the typhoid toxin directly stimulates to proliferation the endothelial cells, which then devour their offspring, the lymphocytes, and the red corpuscles.

I have suggested as another explanation that the typhoid bacillus produces a lymphocytotoxin and a haemolysin, and that the proliferation of the fixed cells is partly compensatory and partly for the increased production of macrophages. We already know that this bacillus generates a haemolytic agent, and we also know that one of the effects of injection of haemolysins is to increase greatly the number of macrophages containing red corpuscles in the spleen.

Through the kindness of Professor Flexner I have had the opportunity of studying the extraordinary changes produced in all the lymphatic glands and in the bone marrow of rabbits by injections of lymphotoxic or myelotoxic serum obtained by treating a goose with lymphatic or marrow tissue of the rabbit. One of the most striking effects of this poison for lymphocytes and other leucocytes is the very extensive proliferation of the reticulum cells in the lymphatic nodes and of the marrow cells. In the light of these observations it is clear that a positive demonstration of the production of a lymphotoxin by the typhoid bacillus would materially advance our understanding of the morbid anatomy of typhoid fever. Another lesion of this disease, only second in importance to those mentioned, is the occurrence of plugging of the small vessels. Dr. Fisher, in my laboratory, has recently shown that such thromboses are produced by the experimental inoculation of rabbits with the typhoid bacillus. I have already pointed out that many of these plugs are agglutinative thrombi.

Of course infectious diseases other than typhoid fever could also be cited, did time permit, as equally forcible illustrations of the aid which pathology may reasonably expect from more precise knowledge of the bacterial cellular poisons. It is probable that such knowledge will lead to improvements in the quality for therapeutical purposes of the so-called bacteriolytic serums, some of which, as now prepared, are not so wholly devoid of antitoxic properties as is often represented. We may also anticipate from investigations of the character indicated much light upon one of the most puzzling of bacteriological problems—the localization of bacteria in disease. Toxic lesions and the plugging of small blood vessels are certainly often of decisive influence in determining this localization, as has been demonstrated especially for the staphylococcus pyaemias by Muscatello and Ottaviano.[22]

The toxins to which I have chiefly directed your attention in this lecture are those produced by bacteria. But, as already pointed out, we now know that the animal body has the power to produce specific poisons directed not only against invading bacterial cells, but also against all sorts of foreign cells. Following the discovery by Belfanti and Carbone in 1898 of this capacity in relation to injections of blood a wholly new domain of biology has been opened to experimental research. Attention has been withdrawn for the moment to a considerable extent from the bacterial toxins and concentrated upon the animal cytotoxins. Here new facts and conceptions of absorbing interest have been disclosed in an abundance and with a rapidity which are simply bewildering.

It was my original design to include in this lecture a consideration in some detail of these animal cytotoxins, but so much time has been occupied with other aspects of the subject that I am compelled to abandon this intention. This is perhaps less to be regretted, inasmuch as I understand the main purpose of these lectures to be the presentation of applications to medicine and surgery of scientific discovery, and it is precisely this side of the recent work on animal cytotoxins which seems to me in the main not yet ripe for profitable discussion on this occasion. It is true that facts of much scientific and practical interest have been discovered by the investigations, initiated by Shattock and by Grünbaum, followed by Landsteiner, Ascoli, Eisenberg, Kraus and Ludwig and others concerning the isoagglutinative and isolytic properties of human serums in health and in disease.

[22] Muscatello and Ottaviano: Virchow's Archiv, 1901, CLXVI, p. 212.

But the really great practical questions in this domain relate to the production of autocytotoxins in the human and the animal body. What is the nature of that very efficient regulatory mechanism underlying the horror autotoxicus (Ehrlich) which prevents either the action or the formation of autocytotoxins in consequence of absorption of our own degenerated and dead cells? Can this protective mechanism be overthrown by pathological states and self-generated cellular poisons become operative in the causation of anaemias, haemoglobinurias, chronic interstitial inflammations, uraemia, eclampsia, epilepsy and other diseases? To these and similar important questions the existing experimental data seem to me too insufficient and inconclusive to furnish any decisive answer at present. I share, however, the hope and belief of many that here is a field for exploration which, although surrounded with many difficulties, gives promise of discoveries of a far-reaching and important nature. I anticipate that some future Huxley lecturer will find in this realm a fascinating theme.

In this connection may be mentioned the great pathological interest pertaining to the recent investigations of Jacoby, Conradi and others on the phenomena of self-digestion or autolysis of inflammatory exudates and necrotic material within the living body. One can readily convince himself of the energetic action of autolytic ferments by the simple experiment of placing a piece of fresh pneumonic lung in the stage of gray hepatization under chloroform and noting the rapid solution of the exudate, in contrast with the absence of this process in earlier stages of the disease. Conradi finds that bactericidal substances, to which he attaches much importance, are produced in tissues and cellular exudates undergoing autolysis.

Although my theme relates especially to the bearing of studies of immunity on pathology, it is hardly necessary to say that these studies were primarily undertaken to elucidate the great problems of predisposition and resistance to disease, and that in this field they have borne their richest fruits. It is especially gratifying to note the close convergence of the two doctrines of immunity, the cellular and the humoral, brought about by these recent discoveries. On the one hand the phagocytic school, represented so brilliantly by Metchinkoff and his coworkers in the Pasteur Institute, recognize and apply to the fullest extent in the explanation of the acquired immunity the cytolytic principles based upon the cooperative action of intermediary bodies and complements. On the other hand the humoral school, led by our German *confrères,* which has been so fruitful in results of the greatest scientific and practical value, recognize the cells, and especially the leucocytes and other cells of the blood-forming organs, as the immediate source of the protective substances. There are many differences in details, especially in terminology and in interpretation, which make the divergence seem greater than it really is. The essential difference between the two schools concerns the place where the two forces, intermediary body and complement, unite. All are agreed that the intermediary body exists free in the blood plasma, but Metchnikoff strenuously insists, especially on the basis of Gengou's experiments, that the complement or cytase is within the leucocytes, from which it is not secreted but can be liberated only through damage to these cells. This question certainly needs further investigation before it can be regarded as settled.

The deeper insight which we have recently gained into the nature of the forces concerned in immunity makes especially desirable the systematic study of the blood and other fluids of human beings in health and in disease with reference to their content of specific antibodies, particularly of the bactericidal substances. It can scarcely be doubted that knowledge of this kind will be in various ways of practical value to the physician and surgeon. The simplest procedure, and the one hitherto generally adopted, is the estimation of the bactericidal power of the blood serum *in toto*. For this purpose Professor Wright[23] has devised an ingenious method which in his hands has furnished extremely interesting information concerning variations in the bactericidal power of the blood as regards the typhoid bacillus in health, under the influence of fatigue, in the course of typhoid fever and after antityphoid inoculations. The older methods, however, while not without value, do not inform us of the total content of the blood in immunizing substances, and have led to very discordant results, particularly as to the influence of infection upon the bactericidal power. Thus Conradi[24] finds, in opposition to most previous experimenters as well as to the later results of Wilde, that infection with the anthrax bacillus does not at any stage influence materially the bactericidal properties of the blood.

A useful and readily applicable method for the determination separately of the intermediary bodies and of the complements of human serum is urgently needed. When one takes into consideration the plurality of complements and of intermediary bodies, the fallacies of interpretation which may arise from failure to take account of anti-complements, of anti-immune bodies, of complementoids, of amboceptoids, of deviation (*Ablenkung*) of complements, and other principles in this complicated subject, it is clear that the problem is not an easy one.

Notwithstanding these difficulties, work has already begun along these new lines, and has led to interesting results. We know that the content of the blood in specific antibodies, and especially in complements, varies in significant ways under diverse conditions, as in infancy and in adult life, in health, in different states of nutrition, under the influence of fatigue, of inanition, of pain, of interference with respiration, of alcohol, and in disease. The infant comes into the world with protective antibodies in the blood smaller in amount and less energetic than those possessed by the healthy adult. It is an important function of the mother to transfer to the suckling through her milk immunizing bodies, and the infant's stomach has the capacity, which is afterwards lost, of absorbing these substances in an active state. The relative richness of the suckling's blood in protective antibodies, as contrasted with the artificially fed infant, explains the greater freedom of the former from infectious diseases.

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[23] A. E. Wright: Lancet, 1898, I, p. 95; 1900, II, p. 1556; 1901, I, pp. 609 and 1532, and 1901, II, p. 715.

[24] Conradi: Zeitschrift für Hygiene, 1900, XXXIV, p. 185; 1901, XXXVIII, p. 411.

The important question of the influence of preexistent disease in predisposing to infection has been brought nearer to a solution by recent studies of immunity. Schütze and Scheller[25] have demonstrated that, while the normal rabbit promptly regenerates the complements used up in consequence of the injection of haemolytic serum, a rabbit infected with the hog-cholera bacillus has lost this capacity. My former pupil, Dr. Longcope, has kindly placed at my disposal the unpublished results of an investigation which he is making under Professor Flexner's direction at the Pennsylvania Hospital of the intermediary bodies and the complements in human blood in different diseases. Colon and typhoid bacilli are used as the tests, as, unless one accepts Bordet's doctrine of the unity of complements, it is more important for the study of problems of infection to determine bacteriolytic rather than haemolytic antibodies. One of the earliest results of the systematic bacteriological examinations which we make at all necropsies at The Johns Hopkins Hospital was the recognition of the great frequency of terminal infections, formerly often undetected by the clinician, in chronic diseases, particularly of the heart, the blood vessels, and the kidneys. Dr. Longcope finds, although not regularly, still in many cases of these diseases a marked reduction in the quantity of complements, which may amount to a total loss of the colon complements. The analysis of the cases brings out unmistakably a definite relation between this loss of complement and the predisposition to infection.

The study of a series of acute infections, chiefly of a surgical nature, shows that in the course of the infection complements are being constantly used up and regenerated, and that at any given time there may be an excess or a reduction of the bacteriolytic power of the blood. Thus far it has been found impossible in these acute infections to attach any prognostic significance to the amount of complement or of bacteriolytic power, nor could any definite relation be determined between the leucocyte count and the content of complements.

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[25] Schütze and Scheller: Zeitschrift für Hygiene, 1901, XXXVI, pp. 270 and 459.

Although we have traversed, gentlemen, in this lecture a path which I fear has seemed to you a long and winding one, I am conscious that I have been able to point out the features of the prospect only imperfectly and incompletely. The extent and the richness in details have been embarrassing. I trust, however, that I have been able to indicate in some measure the great interest and importance to biology, to physiology, to pathology, to every department of medical science and art of investigations which have led to a deeper insight into certain manifestations of cellular life. What has been conquered by these investigations is simply a bit of new territory pertaining to the intimate life of the cells, and we find here, as whenever we are able to penetrate deeper into this life, that there comes a flood of new light into every department of biology. The researches on immunity, which to some of short vision once seemed to threaten the foundations of cellular pathology, have served only to strengthen them. These researches, which have already led to the saving of thousands of human lives, and will lead to the saving of untold thousands more, have been carried on by the experimental method, and can be conducted in no other way. This method is as essential for the advancement of medical science as for that of any of the natural or physical sciences. To restrict unnecessarily and unjustifiably its use is nothing short of a crime against humanity. It is an evidence of the robust vitality of English physiology and medicine that in spite of unwarrantable obstacles thrown in their path their contributions to science in recent years have been so numerous and so important. The influence of English thought and action is great with us in America. See to it, my colleagues and men of science in the British Isles, that you retain for yourselves and hand down to your successors, at least without further impairment, the means of promoting medical knowledge and thus of benefiting mankind.

TRAUMATIC CEREBRAL ABSCESS, WITH BACTERIOLOGICAL EXAMINATION[1]

E. M., negro, aged 26. Ten days before admission patient had been struck on the left side of the forehead, about 1½ inches above the upper border of the orbit, with a grubbing hoe. No particular symptoms developed until the day before admission, when he had what was called a fit. At the time he was working on board of a boat and fell overboard with loss of consciousness. Upon admission there existed a little below and to the outer side of the left frontal eminence a suppurating wound, beneath which was recognized a depressed fracture of the skull. The depressed fragments were elevated and removed. Beneath them the dura mater appeared intact. The patient's general condition after the operation seemed good for several days. He then became inclined to sleep much and seemed dull. His mind and memory were somewhat impaired, but he would answer questions rationally and sometimes he was much brighter mentally than at others. No distinct paralysis could be recognized. He presented no urgent symptoms until a few hours before his death, when he complained of headache and vomited. He passed into a condition of stupor and died somewhat suddenly. Death occurred 64 days after the injury, 50 days after the operation.

At the post mortem examination, which was made soon after death, with the exception of general pulmonary oedema and moderate swelling of the spleen, the only important lesions were of the skull and brain.

On the left side of the forehead, a short distance below the border of the hair, is a semicircular white scar, 9 cm. in length, healed throughout except over an ulcerated, granulating surface of 1.5 cm. from the median line near the border of the hair. This ulcerated surface corresponds to a round defect in the frontal bone filled with firm gray cicatricial tissue. Upon removing the calvaria the dura mater is found firmly adherent around the margins of this trephined opening and to the cicatricial tissue filling it. The dura is also adherent to the pia-arachnoid membrane of the left frontal lobe over an area 3 cm. in diameter, which corresponds to the hole in the bone. Elsewhere the dura and the pia-arachnoid membranes are free from

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[1] Report of presentation of a pathological specimen and report before The Johns Hopkins Hospital Medical Society, Baltimore, November 3, 1890.

Johns Hopkins Hosp. Bull., Balt., 1891, II, 141-142.

any inflammatory reaction, there being no acute meningitis. The convexity and the median surface of the left frontal lobe in its anterior part appears swollen, the convolutions somewhat flattened, the pia-arachnoid over this part paler than elsewhere, and upon palpation this part of the brain is softer than the rest and affords a distinct sense of fluctuation. These changes do not extend so far back as the central convolutions. Upon incision the left frontal lobe is found to be the seat of an abscess from which about 50 c. c. of creamy, opaque, green pus escapes. The abscess is oval in shape and measures 5.5 cm. in its longest diameter. It occupies the centrum ovale of the anterior part of the frontal lobe, its posterior margin lying 5 cm. in front of the fissure of Rolando. The gray matter is not involved in the abscess except at one point where the abscess extends to the surface of the brain, and here its wall is formed by the thickened pia-arachnoid membrane. This corresponds to an area 1 cm. in diameter where the dura mater, as mentioned above, is adherent to the pia-arachnoid membrane on one side, and to the margins of the hole in the skull and the overlying cicatricial tissue on the other. The spot where the gray matter is thus destroyed by the abscess is in the middle frontal convolution on the convexity 3 cm. behind the anterior pole of the brain, and 2.5 cm. from the median border. The wall of the abscess is formed by a bluish, slate-colored, membranous capsule, averaging 2 mm. in diameter, covered on its inner surface with opaque, greenish white, adherent material, and surrounded externally by very soft, apparently disintegrated, moist, white brain substance for a distance of 1 cm., the softening gradually merging into normal cerebral substance. The capsule of the abscess is of firm consistence, and readily separates from the surrounding softened brain substance. The pus within the abscess has a deep green color and a very decided, offensive odor of sulphuretted hydrogen. The basal ganglia and internal capsule are normal.

On the surface of the right middle temporal convolution, near the angular gyrus, is a superficial defect about 1 cm. in extent, of a brownish color covered by pigmented brownish pia-arachnoid. This defect is free from any acute inflammatory reaction and is doubtless the result of contrecoup at the time of injury. On the inner surface of the dura mater covering the base of the frontal lobe are several thin yellow patches 0.5-1 cm. in diameter, evidently the result of slight haemorrhage. The later ventricles contain a slightly increased amount of serum, which is a little turbid. The vessels are normal and there are no other lesions in the brain than those described.

Fresh cover-slip preparations from the pus in the cerebral abscess show a moderate number of cocci. These cocci occur in pairs and in small clumps.

They present the appearance of ordinary *Staphylococcus pyogenes.* None were found inside of cells. The cover-slip preparations were made immediately upon the removal of the brain and without any chance of contamination. Cultures were made from the pus, both by smearing a considerable quantity of the pus over the surface of slanting agar, and by making a set of roll agar cultures. These tubes were kept at body temperature in the thermostat and remained absolutely sterile.

There are several points of interest in connection with this case of cerebral abscess. The direct connection of the injury with the development of the abscess is clear, a straight path being made thereby for the invasion of microorganisms from without, a path clearly evident at the post mortem examination. The absence of marked symptoms and their obscure character is explicable by the seat of the abscess in the white matter of the anterior part of the frontal lobe. Had the nature of the affection been recognized a more favorable case for surgical interference could hardly be imagined, in view of the absence of acute meningitis, the strictly localized character of the lesion and the cicatricial adhesions between the wall of the abscess and the overlying tissues at the spot corresponding to the defect in the bone.

The finding of micrococci, presumably the ordinary pyogenic staphylococci, by microscopical examination of the pus and the failure to obtain them in cultivation are points of especial interest.

It can hardly be doubted that the micrococci which were found were concerned in the causation of the abscess. Whether they were the only organisms concerned is uncertain. No other species was found by microscopical examination, but the distinct odor of sulphuretted hydrogen possessed by the pus may point to the existence of some other organism. Still it is possible that the ordinary pyogenic staphylococci are capable of producing that decomposition of the chemical substances composing the brain which yields sulphuretted hydrogen, although in abscesses caused by them in other parts of the body no odor of sulphuretted hydrogen is present. In the long interval which had elapsed after the injury (64 days) the micrococci had evidently died, as is shown by the results of the cultures from the pus. It is not uncommon in very old suppurative processes to obtain negative results with cultures from the pus, and the present case shows conclusively that in such instances the negative result should not be interpreted as evidence that the suppuration was not due to bacteria for the bacteria which caused the disease may have perished.

CONDITIONS UNDERLYING THE INFECTION OF WOUNDS[1]

INCLUDING A DISCUSSION OF DISINFECTION WITH REFERENCE TO TREATMENT OF WOUNDS, OF THE RELATION OF BACTERIA TO SUPPURATION, OF THE RESISTANCE OF TISSUES TO THE MULTIPLICATION OF BACTERIA AND OF THE EFFECTS OF ANTISEPTIC AGENTS ON WOUNDS

That the presence of certain kinds of bacteria is an essential condition of wound infection is a fact so well established, and so generally recognized, that no discussion as to this point is likely to arise in this assembly. The practical results of the application of this doctrine to the management of wounds are the most eloquent testimony to its lifesaving truth. The recognition of this truth, even before its complete demonstration, and the introduction of methods of wound treatment based upon it, will remain the immortal merit of Lister, even although every detail of his treatment be replaced by measures found by riper experience to be better suited to the purpose.

The simple conception which was the basis of early antiseptic procedures in surgery—that a wound to which bacteria gain access becomes infected in the same way as a sterilized infusion of meat undergoes putrefaction when a single suitable germ enters—has been greatly modified. Furthermore, it is found that the traumatic infections present their own peculiar problems, which must be studied by themselves, and cannot be solved by analogies drawn from observations of other specific infections, such as anthrax and the septicaemias of the lower animals. The study of wound infections involves the consideration of many varying and often complicated factors, relating both to the agents of infection and to the individual exposed to infection.

Without consuming time with any historical review, I shall proceed at once to indicate some of the questions which are of especial importance, and which may be profitably considered in this discussion.

What are the microorganisms concerned in the infection of wounds, and how do they act?

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[1] An address before the Congress of American Physicians and Surgeons, Washington, D. C., September 22, 1891.

Tr. Cong. Am. Phys. & Surg., N. Haven, 1892, II, 1-28.

How are we to explain the great differences in the effects produced by the pyogenic bacteria, their apparent harmlessness under some conditions, their fatal virulence in others?

What are the ways by which bacteria gain access to a wound?

How often are bacteria to be found in wounds treated antiseptically or aseptically? What are the characters of these bacteria and whence do they come?

What are the best means of surgical disinfection?

No attempt can be made on this occasion to treat exhaustively a single one of these questions. My aim will be to present a brief survey of our knowledge, as well as of the defects in our knowledge, concerning some of the more important questions; to offer some results of personal observation and experiment, and to indicate in conjunction with the co-referee the lines which this discussion may follow. It does not seem necessary, indeed it would be presumptuous before this audience, to dwell in detail upon established facts which are the common property of all who are interested in the subject.

So far as personal observations and experiments are concerned, I may say that these have been made in The Johns Hopkins Hospital and Pathological Laboratory, and that I am indebted to several of my colleagues and coworkers there for work which they have done either independently or under my supervision. I take this opportunity of acknowledging especially the assistance of Drs. Halsted, Abbott, Ghriskey, Robb, and Howard.

This paper will relate only to the ordinary inflammatory and suppurative traumatic affections, and not to specific wound infections, like tetanus, diphtheria, and hospital gangrene.

It would be wearisome on this occasion to consider or even enumerate all the bacteria which have been found in suppurative and other traumatic inflammatory processes, so that in this connection I shall touch only upon a few points drawn chiefly from our own experience.

We have found *Staphylococcus pyogenes aureus* far more frequently than any other species of bacteria in furuncles, abscesses, osteomyelitis, and other forms of suppuration. The prevalence in Strassburg, according to Levy, of *Staphylococcus pyogenes albus* in all kinds of suppurative inflammation (excepting furuncles), raises the question whether there may not be differences in different regions as regards the relative frequency of the various kinds of pyogenic cocci.

We have, however, met a white staphylococcus in a certain class of cases with great frequency, viz., in small stitch abscesses, and in the slighter grades of inflammatory disturbance of wounds treated antiseptically or aseptically. This coccus has been often seen by Bossowski and others in the same class of wounds, and has been hitherto identified with *Staphylococcus pyogenes albus* of Rosenbach, but we have some hesitation in so regarding the micrococcus which we have found in the cases mentioned. *Staphylococcus pyogenes albus* is generally described as not differing essentially from the aureus, except by the absence of the yellow color in the cultures. This coccus, however, differs from *Staphylococcus pyogenes aureus* in much greater slowness of liquefaction of gelatin and of coagulation of milk, and in far less virulence when inoculated into the circulation of rabbits. It may be an attenuated form of *Staphylococcus pyogenes albus.* Until this is *settled* I propose to call it *Staphylococcus epidermidis albus,* as it is an almost constant inhabitant of the epidermis, as will be apparent when I come to speak of the bacteria in aseptic wounds. I shall only add here that this coccus may be present in graver suppurative inflammations, but then it has been nearly always associated with some other pyogenic organism, or has assumed the form of typical *Staphylococcus pyogenes albus.*

It is, perhaps, not out of place to remark that the diagnosis of *Staphylococcus pyogenes albus* in cultures from abscesses and other suppurations should not be made too hastily. It may require several days before colonies of the aureus assume a distinct yellow color. Cultures upon potatoes are particularly favorable for the speedy development of this color. There may be marked differences in the rapidity with which different colonies of the aureus from the same source and in the same plate or roll cultures turn yellow.

The efforts to differentiate into distinct species the pathogenic streptococci have thus far met with little success, so that the weight of opinion favors the view that the streptococcci of erysipelas, of phlegmonous inflammations, of septicaemia, of puerperal fever, and of various forms of angina, belong to one and the same species. In conformity with the results of most investigators I have been unable to determine any decisive differences between *Streptococcus erysipelatos* and *Streptococcus pyogenes.* Von Lingelsheim has recently divided the streptococci into two groups: one nonpathogenic, called *Streptococcus brevis,* and the other pathogenic, *Streptococcus longus.* Among the points of distinction which he gives there is one which I have not found constant, viz., the invisible growth of all pathogenic streptococci upon potato. The behavior is usually as stated by Von Lingelsheim, but I have isolated from a case of phlegmonous cellulitis, and from one of septicaemia, a streptococcus presenting a distinct grayish white growth upon potato. Von Lingelsheim made no use of one of our most valuable differentiating media, viz., sterilized milk colored with litmus. In this medium common *Streptococcus pyogenes* produces a very firm coagulum with separation of nearly colorless serum, as has been pointed out by

E. Fränkel. I have from different sources isolated pathogenic streptococci which do not coagulate milk, although they change the color of the litmus to a lilac pink, indicating the production of a certain amount of acid.

While we must admit that all inflammatory affections caused by the pyogenic staphylococci may be produced also by streptococci, and, with the probable exception of erysipelas, the reverse also holds true, nevertheless we are not prepared to accept the statements of Levy and some other recent writers that the character of the microorganisms, whether staphylococci or streptococci, has no influence upon the symptomatology of the disease. In conformity with most investigators, we have found the staphylococci prone to form circumscribed areas of suppuration, whereas the pyogenic streptococci have a tendency to produce spreading phlegmons with lymphangitis and a wide zone of surrounding redness and inflammatory oedema. It is the custom in our gynecological wards to isolate the operative cases in which streptococci are found at the time of the operation in the seat of the disease, or subsequently in the wound.

Of other pyogenic cocci we have found *Staphylococcus pyogenes citreus* twice in abscesses, *Staphylococcus cereus albus* three times in combination with other bacteria in suppurating wounds, *Diplococcus pneumoniae* once in a fibrinous peritonitis, and *Micrococcus tetragenus* once, together with *Staphylococcus aureus* in an abscess of the lung.

The list of bacilli which may be concerned in suppurative and other inflammatory affections is much longer than was formerly supposed, and is likely to be still further extended. Those which we have chanced to encounter are: *Bacillus pyogenes foetidus,* twice in abscesses containing bad-smelling pus; *Proteus zenkeri,* once in pure culture in an ovarian abscess with purulent salpingitis; a bacillus apparently identical with *Bacillus emphysematis maligni* of Wicklein, once in an emphysematous cellulitis of the arm; a bacillus apparently identical with *Bacillus enteritidis* of Gärtner, in the brain and other organs of an infant three months old with cerebral abscess and meningitis following operation for imperforate anus; the typhoid bacillus, once in pure culture in an osteomyelitis of the ribs following typhoid fever; a hitherto undescribed bacillus, rapidly liquefying gelatin and characterized by rapid solution or peptonization of milk, without coagulation, once in peritonitis; bacilli which could not be cultivated in our ordinary culture media, in three cases of suppurative inflammation; and *Bacillus coli communis,* fifteen times.

As these bacillary infections are far less common than those caused by micrococci, I shall take some other occasion to describe them, and here shall ask your attention only to one group, viz., those associated with *Bacillus coli* *communis*, as they have been relatively common in our experience and present some points of especial interest.

The first observation of *Bacillus coli communis* in connection with wound infection made by Tavel, in 1889. This has been followed by a few isolated observations of this organism either in the unchanged organs of the body or in suppurations, until recently A. Fränkel reports its presence in nine out of thirty-one cases of peritonitis. I first came across this bacillus in the organs of the body in 1890, in a case of multiple fat necrosis with pancreatitis, which I reported to the Association of American Physicians. As in this case diphtheric colitis existed, it seemed probable that the lesion of the intestine opened the way for the entrance into the circulation of this inhabitant of the healthy intestinal canal. This view subsequent experience has confirmed, for I have already isolated in pure culture from the internal organs *Bacillus coli communis* in twenty-five autopsies where some distinct lesion of the intestinal mucous membrane existed, such as ulceration, diphtheritis, haemorrhage, traumatic injury, and I have almost uniformly failed to find it outside of the intestine when no demonstrable lesion of the mucous membrane existed. I am therefore prepared to say that this bacillus is an extremely frequent invader in intestinal disease, although I have no evidence to offer that it does any harm except under certain conditions. The autopsies were in nearly every case made within a few hours after death, once in less than one hour. Moreover, the colon bacillus does not invade the blood and organs in the process of post mortem decomposition.

The cases in which we have found the colon bacillus under circumstances pointing to its pathogenic action have been as follows: perforative peritonities, four cases; peritonitis secondary to intestinal disease without perforation, two cases; circumscribed abscess, three cases; and laparotomy wounds, six cases.

Its presence, several times in pure culture, in laparotomy wounds treated aseptically, although apparently not a source of serious trouble, was not a matter of indifference. It was generally accompanied with moderate fever and a thin, brownish, slightly purulent discharge, of somewhat offensive, but not putrefactive odor. The smooth and rapid healing of the wound was interfered with. In some of the cases there was evidence of intestinal disorder; in others this was not apparent, and infection from without could not be excluded.

For the purposes of the present discussion, perhaps the chief interest of our observations concerning the colon bacillus is that they furnish an illustration of the possible predisposition to infection afforded by intestinal lesions, and also give an example of the much disputed auto-infection.

Of suppurative inflammations which we have examined bacteriologically with negative result, may be mentioned abscesses in which the bacteria were presumably dead, several hepatic abscesses caused by *Amoeba dysenteriae,* and many cases of suppurating buboes and of pyosalpinx, doubtless caused by the gonococcus.

From the foregoing brief summary, mainly of our own limited experience, it is clear that the bacteria which may be concerned in surgical infections are many, but the pyogenic cocci of Ogston, Rosenbach, and Passet far outrank in frequency, and therefore in importance, all other bacteria combined.

In view of the more practical matters to be considered, we cannot tarry long over the theories of suppuration, interesting as just this part of the subject is at the present time.

There are very good reasons to believe that the process of suppuration serves a useful purpose, and is one of the most important and efficient weapons employed by nature in combating invading microorganisms. Man is not subject to those forms of septicaemia, common in many lower animals, in which enormous numbers of bacteria are present in the blood; but, on the other hand, he is particularly subject to suppurations and other localized inflammations The same microorganisms, for instances *Diplococcus pneumoniae* and *Bacillus anthracis,* which in many of the lower animals invade the blood in large number, cause in man localized inflammations. In the experimental septicaemias of lower animals there is an unmistakable relation between the extent of the local reaction at the point of inoculation on the one hand, and the duration of life and number of organisms present in the blood on the other. The more extensive and intense the local reaction, the fewer, as a rule, are bacteria found in the blood and the longer the duration of life. There are two varieties of the swine-plague bacillus, the one kills rabbits in twelve to eighteen hours with trifling local reaction and large number of bacilli in the blood, the other produces extensive purulent infiltration around the site of subcutaneous inoculation and permits the animals to live for several days, and at autopsy very few bacteria are found in the blood. In a susceptible animal the virulent anthrax bacillus produces an acute septicaemia, the attenuated bacillus a local abscess. There is even experimental evidence that if inoculation of a susceptible animal with the virulent anthrax bacillus be speedily followed by inoculation at the same point with a pyogenic organism so that suppuration soon ensues, the development of anthrax septicaemia may be warded off and the animal recover. It seems reasonable to infer, when the same organism produces in one animal a rapidly fatal septicaemia without local reaction and in another species only a local abscess, that the latter animal is protected by the barrier of suppuration. As is well known, the fatal infections from wounds received at post mortem examinations are not generally those where marked local reaction with suppuration appears at the seat of the often trifling injury.

Exactly how abscess formation checks the invasion of bacteria we do not know. That bacteria may die, often in a short time, in pus both within and outside of the body, has been demonstrated. The leading theories are these: leucocytes and other cells act as phagocytes, taking into their bodies the bacteria and killing them;—the wall of leucocytes and other cells at the margin of an abscess acts as an obstacle to the passage of the bacteria into the surrounding tissues;—pus contains chemical substances injurious to bacteria or antidotal to their toxic products;—the bacteria starve in pus, not being able to assimilate such concentrated food. Something can be said in favor of each of these theories, more in favor of some than of others, but none is proven and we cannot stop to discuss them here.

That pyogenic bacteria set up suppuration by chemical substances produced by them, seems to be proven. The zone of necrosis around these bacteria which can be demonstrated as the first effect of their lodgment in the tissues points to such chemical action. Moreover, from cultures of pyogenic cocci several chemical products have been obtained capable of causing suppuration. The most interesting observations here are the recent ones of Buchner and his pupils, who have found the proteid constituents of many bacteria capable of producing suppuration. These bacterioproteins of Buchner possess in a remarkable degree the property of positive chemotaxis, that wonderful quality by which certain chemical substances influence the leucocytes to migrate from the vessels and move actively toward them. A flood of unexpected light seems destined to come from the studies of chemotaxis to illumine many dark problems in pathology.

Since we know that the chemical products and constituents of certain bacteria are the direct agencies in the production of suppuration, it is less surprising to find that various other chemical substances are likewise able to cause suppuration. But important as this fact is for the theory of suppuration, the demonstration that these other chemical irritants, such as turpentine, nitrate of silver, etc., can cause suppuration has no especial bearing upon the ordinary suppurations in human beings.

We come now to the consideration of a division of our subject beset with difficulties at every turn, but one no less interesting to the surgeon than to the bacteriologist.

How are we to explain the extraordinary variability in the effects produced by the pyogenic cocci? The specific infectious agents with which we first became familiar, the bacteria of the natural and of the experimental septicaemas of lower animals, are nearly constant in the effects which they produce when inoculated in small quantity into susceptible animals. Not so with the pyogenic cocci. Here we meet the most puzzling differences. We find the same coccus in the most insignificant epidermal pustule which we find in a dangerous phlegmon, in osteomyelitis, in pyaemia, in septicaemia, in acute ulcerative endocarditis. The manifold varieties of puerperal fever, which we now know to be a typical wound infection, may be caused by apparently the same streptococcus, producing a mild endometritis, a pelvic abscess, localized or general peritonitis, pyaemia or the most virulent and rapidly fatal septicaemia.

If we seek an explanation of these things by experimentation upon animals, we find that in order to get any positive effects at all it is often necessary to introduce enormous quantities of our cultures, containing vastly more bacteria than we can suppose to be concerned in the primary infection in human beings. If we inoculate smaller quantities, these can often be disposed of by the animal without any manifest symptoms.

It must be confessed that we stand here before problems many of which still await solution, but it will not do to pass them by on this occasion without some discussion, although time will permit me to touch upon only some of the salient points.

It is well to bear in mind that the inflammatory infections of wounds do not represent specific morbid entities in the same sense as do anthrax and typhoid fever, for example. The latter diseases are caused by a single specific germ and no other, and present a definite and characteristic clinical and anatomical picture, whereas the former correspond to the reaction of the body toward a great variety of noxae and offer manifold variations in symptoms and lesions.

The quantity of a culture of *Staphylococcus aureus* required to produce suppuration is not of the same for all tissues and all parts of the body. A mere trace, a fraction of a drop, of a dilute suspension of the yellow staphylococcus in salt solution when injected into the rabbit's eye will set up a suppurative inflammation. It takes a larger amount of such a suspension to produce an abscess in the loose subcutaneous tissue of the back than in the dense tissue of the ear of a rabbit or in muscular tissue. Enormous quantities, five to ten cubic centimeters and more, of the suspension can be injected often without effect into the normal peritoneal cavity of rabbits and dogs, as has been shown by the well known experiments of Grawitz and his pupils, confirmed by several other investigators.

It is probable, as claimed by Grawitz, that this difference in the behavior of different tissues depends in large part upon the rapidity with which the injected material, more particularly the toxic substances, are absorbed. But we must also reckon with a predisposition apart from this in the normal tissues, for the introduction even of very large numbers of staphylococci into the circulation of rabbits is followed by the formation of abscesses only in certain situations, mainly the kidneys, heart and certain muscles, although we must suppose that the cocci have been carried to all parts of the body.

That there are variations in the virulence of different cultures of the pyogenic cocci is admitted by most writers and may be inferred from the discrepant results of various experimenters as regards the amount of the culture required to produce suppuration. These discrepancies cannot be explained wholly by different conditions of the experiments. We have found no especial difference in the result, for example, whether we introduced into the peritoneal cavity of dogs cultures of *Staphylococcus aureus* according to Grawitz's method, or according to Pawlowsky's method, or by a small laparotomy wound. By one as well as by another method we are able to inject 5 c. c. and often more of a suspension in salt solution of the yellow staphylococcus without any appreciable effect.

In order to test this matter of varying virulence, I have injected into the ear veins of rabbits bouillon cultures, forty-eight hours old, of *Staphylococcus aureus* obtained from many different sources. Considerable variations in virulence were found to exist both in the original cultures and in the successive generations of the same culture. The most virulent cultures were one from a beginning furuncle and one from a fresh peritonitis. The injection of 0.1 c. c. of these cultures caused the death of the rabbit in twenty-four hours with a large number of staphylococci in the blood and many necrotic foci in the kidneys. After the second generation the virulence was lessened. From a case of hypertrophic cirrhosis of the liver without suppurative complications a moderate number of colonies of *Staphylococcus pyogenes aureus* were found in cultures made from the liver. 1.5 c. c. of bouillon cultures, forty-eight hours old, of this staphylococcus were injected without any effect into the ear-veins of two rabbits, the animals being still alive eight months after the injection. These extremes are met only exceptionally. In the great majority of cases we have found the injection of 0.2 to 0.3 c. c. of bouillon cultures sufficient to kill the animal in four to seven days with the usual abscesses in the kidneys and heart and occasionally elsewhere. As a rule there was no material change in the degree of virulence for many successive generations, but sometimes without any apparent cause there would occur a marked weakening of the virulence either after a few generations or after many.

Our conclusions from these experiments are that while variations in virulence occur and may explain in large part the varying results of different experimenters operating upon animals under similar conditions, they are not sufficient in degree or in frequency to afford adequate explanation of the great differences in the effects of the pyogenic cocci upon human beings, or at least can do so only in part.

How far can we apply to human beings the experiments showing the great tolerance of animals toward the pyogenic cocci? The experimental evidence upon this point is naturally scanty, but such as we possess indicates that man is not equally tolerant, that his tissues respond more readily by suppuration to inoculation of pyogenic cocci. The experiments upon this point of Garré, Bumm, Bockhart, Fehleisen, Waterhouse—and these names should not be mentioned without expressing the debt of gratitude which science owes to them—are too well known to necessitate here any statement of their results. But while these experiments appear to indicate less immunity on the part of human beings toward the ordinary staphylococci of suppuration—a matter, however, which cannot be considered absolutely certain—they have also demonstrated that the difference, if it exists, is only one of degree, and that large numbers of the pyogenic cocci may be inoculated into human tissues without effect. Waterhouse, for example, injected under the skin of the abdomen and of the scrotum 0.25 c. c. of a suspension of *Staphylococcus aureus,* made by mixing one loopful from an agar culture with 5 c. c. of water, and the result was completely negative.

It is true that undue emphasis should not be laid upon the negative results, for there are equally trustworthy experiments with positive result, showing that even small quantities of suspensions of the pus organisms may cause suppuration, both in animals and in human beings.

That there exist bacteria capable of producing abscesses regularly, even when inoculated in very small quantity into animals, is proven by the discovery by Dr. Bolton of a hitherto undescribed bacillus in garden earth. This bacillus, for cultures of which I am indebted to Dr. Bolton, who will hereafter publish its description, produces with certainty localized abscesses when inoculated in small amount into the subcutaneous tissues of rats, mice, and rabbits. When injected into the circulation it causes multiple abscesses in the kidneys, joints, bones, and other situations.

Inasmuch as it is by their toxic products that the pyogenic bacteria do injury, it is not surprising to find that it makes a great difference in the result whether these bacteria enter the tissues already equipped with a reserve force of this poisonous material, or must begin the fight unarmed. There is abundant experimental evidence to show that even in very small number the pus-producing micrococci will cause suppuration if they are accompanied, or soon preceded or followed, in sufficient amount and concentration by various toxic substances which are present in pure cultures of the cocci, and the extent of the inflammation bears a relation to the quality and quantity of these substances. The experiments and researches of many investigators, such as Hankin, Brieger and Fränkel, Buchner, Dirckinck-Holmfeld, Grawitz, Leber, Dubler, Kronacher, Ribbert, have shed light upon this subject. It would seem as if the issues of the battle between the invading micrococci and the tissues depend often upon the first blow; and if the invader can strike this with the aid of powerful weapons which he has forged before he enters, the victory, for the time at least, is with him.

This matter of accompanying toxic substances is probably of great importance in our understanding of the potentialities of the living agents of wound infection as they occur under natural conditions. Here we have to do, not with pure cultures of the pyogenic cocci, still less with those that have been washed in sterilized salt solution or water, but with pus-producing organisms which have come from all sorts of sources, which have been engaged in very different activities, which have been growing under various conditions or have been long dormant, and which are mixed with many kinds of bacteria. There is proof that under some of these conditions the infectious material may possess a degree of virulence with which we are not familiar in our artificial cultures. When a strong, healthy man (I have now in mind such a case in a medical student) dies in a few days from septicaemia caused by the inoculation of a mere scratch on the finger with fluid from a puerperal peritonitis, it is the quality of the infectious material which brought about the fatal result, and not any especial predisposition of the individual. When we find, as has been done, that the peritoneal fluid contains in pure culture *Streptococcus pyogenes,* and that the same organism is present in pure culture in the patient accidentally infected with this fluid, the observation is just as convincing and clear in its interpretation as if the experiment had been made intentionally upon an animal. No number of experiments showing that millions of apparently the same species of micrococcus can be injected in artificial cultures into the peritoneal cavity of dogs and rabbits can do away with the force of such an observation. No surgeon, no obstetrician, however strong may be his trust in the defensive powers of the animal tissues and fluid, would willingly permit the smallest particle of that peritoneal fluid to come into contact with a fresh wound or the uterus after childbirth.

The differences in virulence which have been found to exist between inflammatory exudates from various sources containing pyogenic bacteria are much greater than those observed in the cultures of the same bacteria on artificial media. To explain this, some assume that the differences in virulence pertain to the pyogenic cocci as such, that they are specifically endowed with different biological attributes; while others think that the varying virulence relates not so much to the bacteria as to the character of the toxic substances with which they are associated. In favor of the latter view, which is the one advocated by Bumm, Fehleisen, Chantemesse, and others, may be cited such observations as that of Bumm, who found that the injection into the peritoneal cavity of a rabbit of a quarter of a drop of fluid from a case of acute septic puerperal peritonitis quickly killed the animal with peritonitis, whereas the streptococci artificially cultivated from the same fluid were much less virulent; or the experiment of Fehleisen, showing that a minimal quantity of an artificial culture of *Staphylococcus aureus* added to a little of the clear serum obtained from the germ-free zone of inflammatory oedema around a spreading cellulitis was capable of producing extensive abscesses, whereas the mixture of the same organism with water had no such effect. The question is still an open one, and very likely both factors are concerned.

We have thus far confined our attention to the microorganisms of infection, and have said nothing concerning the conditions predisposing to infection in the exposed individual. Everybody believes in the doctrine of predisposition, some more than others. The tendency at the present time is certainly not to minimize its importance. Predisposition is a term to conjure with. It is often made to explain in a vague sort of way things we do not understand. Nevertheless it is a very real thing, and we cannot pass it by here without notice, even if this must necessarily be very brief. Every surgeon knows that wounds in some persons do much better than in others, and that some kinds of wounds are much more prone to suppurate than others.

The interesting studies upon immunity which have shed so much light upon the nature of predisposition toward some infectious diseases have not as yet cleared up the question of immunity against the pyogenic cocci. We know that the healthy tissues can dispose of a certain number of these cocci under ordinary circumstances, but how they do it we do not know.

An experiment of Roger is suggestive. He found that the streptococci of erysipelas grew as well in the blood serum of rabbits rendered immune as in the blood serum of other animals, but that in the former they lost their virulence. As we now know that the protective influence of the blood serum of immune animals consists quite as much in the power to destroy the poisons produced by bacteria as in the power to kill the bacteria directly—already demonstrated examples of this are diphtheria, tetanus, and septicaemia produced by *Diplococcus pneumoniae*—it is not unreasonable to suppose, in the light of Roger's experiments, that this antidotal capacity of the blood and animal fluids may be one of the means employed by Nature in disposing of the pyogenic cocci. That these cocci are not directly killed by the extravascular blood serum of rabbits, dogs, swine, and human beings, or if at all in very small number, we know. On the other hand, this serum is an excellent medium for their growth. But if the toxic products of the bacteria are destroyed by the fluids of the body, the bacteria can do no harm, and are at the mercy of the tissues, as has been shown by the recent brilliant researches of G. and F. Klemperer on the diplococcus of pneumonia. When we have a clearer insight into the nature of immunity against the pyogenic bacteria, our understanding of the conditions underlying the infection of wounds will be greatly advanced.

I cannot undertake to discuss all the general conditions of the body, such as diabetes, syphilis, alcoholism, anaemia, obesity, typhoid and other fevers, Bright's disease, etc., which have been regarded as predisposing causes of infection with the pyogenic cocci, some of these diseases upon most conclusive clinical evidence. Gärtner has recently brought forward evidence derived from experiments on animals showing that general anaemia and hydraemia render easier the infection with small quantities of *Staphylococcus aureus*, and Ribbert has demonstrated that the presence of toxic products of the same microorganism in the circulating blood favors the development of foci of suppuration, a fact which evidently bears upon the pathology of pyaemia and of some cases of furunculosis, as well as upon the importance of evacuating pus. An instance has already been given of the predisposition to infection afforded by intestinal lesions. In dismissing thus hastily the matter of general predisposition to suppuration, it will not be understood that these few words are a measure of the importance of the subject. I believe that the surgeon cannot be too thorough in the examination, before contemplated operations, of all the important organs and functions of the body, and that, wherever possible, he should endeavor to put the patient into the best possible condition of health before undertaking a severe operation.

Of very immediate practical interest to the surgeon is a knowledge of the various conditions in and about a wound which favor the lodgment and development of pyogenic bacteria. In a general way it may be said that anything which interferes with the integrity of the living tissues in a wound is a predisposing cause of suppuration, in case suitable microorganisms gain entrance. Experiments have shown that the necroses produced by chemical irritants, such as carbolic acid and corrosive sublimate, favor the multiplication of the microorganisms of suppuration. Dr. Halsted has shown that the irrigation of fresh wounds by a solution of corrosive sublimate as weak as 1 to 10,000 is followed by a distinct line of superficial necrosis demonstrable under the microscope.

We are not so well informed as to the influence exerted by blood in a wound. On the one hand, Von Bergmann and most modern surgeons lay the greatest stress upon prompt and careful haemostasis in surgical operations; on the other hand, Schede has revived and more fully developed an old method of treatment, by which a certain class of wounds are permitted to fill with a blood-clot, and he and other surgeons have obtained rapid and aseptic healing by this method. Is this blood-clot, as such, a source of danger in the same sense as dead tissue is? As has already been mentioned, fresh blood serum does not possess any such germicidal power over the pyogenic cocci as it does over the typhoid bacillus and many other bacteria. Not being able to find any direct experimental evidence upon the point, I have, with the assistance of Dr. Howard, made a large number of experiments upon dogs. The operations were done with strict antiseptic or aseptic precautions. In most of the experiments a cavity was chiselled out of bone, and this and the rest of the wound were allowed to fill with a coagulum of blood, the conditions pertaining to similar operations in human beings being observed. The blood-clot, after its formation, was inoculated with a culture of *Staphylococcus aureus,* either by injecting into it a few drops of a fresh bouillon culture or by inserting a platinum loop carrying a bit of the growth on agar. The outcome of the experiments was that the so-called organization of the blood-clot went on as it does in human beings, and the wounds did not suppurate. The staphylococci survived, at least for many days, in the clot, but they did not appear to multiply. This result is in conformity with the experience of Grawitz, who found that the aureus lives for a long time, although it does not multiply, in blood-coagula outside of the body, and that no development of this organism takes place in a solidified mixture of equal parts of nutrient gelatin and blood.

It is not my province to consider the extent of application of the method advocated by Schede to human surgery, nor the advantages of retaining the material employed by Nature in filling up cavities and pockets in fresh wounds as against the insertion of sterilized extraneous material and the obliteration of the dead spaces by deep stitches, which, without great care, are liable to strangulate tissue, produce undue tension, and interfere with the circulation.

Where the healing of the wound by the blood-clot method is not directly purposed, undoubtedly it is important for its aseptic course to check the oozing of blood and to prevent the unintended formation and retention of blood-coagula whose presence is not arranged for by the surgeon in the management of the wound. That loss of blood, as such, predisposes to suppuration has already been mentioned.

In contrast with the negative results of the experiments just mentioned stands a series of positive ones which illustrate the readiness and uniformity with which suppuration of an infected wound ensues which contains masses of tissue strangulated by ligature. These wounds were inoculated with the same cultures which yielded negative results as regards the infection of the blood-clot.

We have made also a large number of experiments upon dogs by ligating portions of the omentum and then injecting cultures of *Staphylococcus aureus,* into the peritoneum. In most of these cases general peritonitis developed, in some localized peritonitis, and in some no peritonitis followed the inoculation.

In order to demonstrate the influence of foreign bodies in favoring suppuration, we inserted into the peritoneal cavity of nine dogs pieces of potato presenting a growth of *Staphylococcus aureus,* and in every instance general peritonitis developed, although in no case was the insertion of similar pieces of sterilized potato followed by peritonitis; and the injection of 1 c. c. bouillon culture of *Staphylococcus aureus* into the peritoneal cavity of twenty-three dogs was not followed in a single instance by peritonitis.

There is a gratifying harmony between the views entertained by bacteriologists concerning the power of the living tissues to overcome a certain number of pyogenic bacteria, and the tendency of the modern surgeon to respect these tissues more and more, not to destroy their vital capacities by the unnecessary application of strong chemical disinfectants, not to bruise them, not to make them too tense, not to strangle them, not to suffer the presence in wounds of spaces and foreign bodies, which remove bacteria from the influence of the living tissues and fluids.

From this necessarily hasty and imperfect survey of this division of our subject, it is apparent that, while there is no reason to doubt that the pyogenic cocci are specific agents of infection, the effects which they produce depend upon a variety of conditions, such as the source, the number, and the virulence of the micrococci, the accompanying toxic substances, the part of the body invaded, the readiness of absorption, the presence of foreign bodies and of pathological products, the general state of the patient, and the condition and handling of the wounded tissues.

In giving due weight to each of these factors one should not forget that the infectious material may exist under natural conditions, in a state capable of causing traumatic infections just as directly, just as certainly, just as independently of predisposition, as infection of a susceptible animal takes place with the anthrax bacillus.

As to the various ways by which pathogenic bacteria may gain access to wounds, there is at the present time general agreement of opinion that the greatest danger is from contact with infected hands, instruments, and other objects. The danger of infection by contact is a lesson which has been learned no less by bacteriological workers in the laboratory than by practical surgeons and obstetricians.

The possibility of infection from the air, insignificant as it may be in comparison with contact infection, cannot be ignored quite as much as some seem inclined to do. *Staphylococcus pyogenes aureus* has been repeatedly found in the dust floating in the air, particularly of surgical wards where there are suppurating cases. *Streptococcus erysipelatos* was found by Von Eiselsberg in the air of a ward containing cases of erysipelas and the streptococci found by Prudden in the air of hospital wards were probably identical with this. When we had more confidence than we now have in the power of chemical disinfectants to destroy all bacteria which might accidentally get into a wound during a surgical operation, it seemed proper to disregard the air as a source of infection, and no less a surgeon than von Volkmann could say, "Auch auf einem Abtritte würde ich dreist operiren wenn die Hände rein wären." A privy, by the way, is not the most dangerous place which he could have selected.

Now that our trust in chemical disinfectants for the purpose named is shaken, and that what is called aseptic surgery is the watchword of the day, I believe that a surgeon who aims at the best will try to have the air of his operating room as free from germs as possible, and will have it so constructed that the floor and walls and all that is in it can be readily cleaned and disinfected. He will regard the influence of currents of air and of commotion in the room in stirring up dust, and will not ignore the value of moisture in laying dust and in keeping it in its place. These suggestions may seem pedantic when the whole tendency of surgery now is to simplify technique and to throw overboard unnecessary ballast, but they appear to me to rest upon bacteriological facts which should not be ignored.

An example has already been given in this paper of auto-infection from the intestinal canal by *Bacillus coli communis*. The not infrequent invasion of the pyogenic cocci in typhoid fever, diphtheria, scarlet fever, and other diseases with lesions of the alimentary tract, are probably explicable partly by these lesions opening a passage for the bacteria into the circulation, and partly by the predisposition afforded by the presence in the body of toxic substances belonging to the primary disease. It is known that the pyogenic cocci are often present in the alimentary canal. For instance, in a case of perforative peritonitis from typhoid fever, recently examined in my laboratory, there were isolated from the peritoneal exudate, not in single but in many colonies on the roll cultures, *Staphylococcus aureus* and *Streptococcus pyogenes,* in addition to the typhoid bacillus, the colon bacillus, and an unidentified bacillus liquefying the gelatin.

Rinne observed in his experiments on dogs that the injection of sterilized putrid fluids together with staphylococci into the peritoneal cavity was followed by suppuration of all open wounds, which otherwise healed kindly, but that subcutaneous wounds were unaffected. The bacteria found in the suppurating open wounds, however, were not those injected, but were derived from the air. This is unquestionably an interesting and important observation, but he goes too far in supposing that such wounds, as well as other *loci minoris resistentiae,* may not become infected from pyogenic cocci in the circulation, as has been demonstrated by the experiments of De Wildt, Waterhouse, and others.

That even under the most unfavorable conditions of general infection a wound may heal by first intention is known to surgeons. In one of our cases, for example, the patient soon after an extensive operation for removal of a cancer of the breast, developed diphtheritic ulcerative dysentery; and at the post mortem examination were found, in addition to the dysentery, pneumonia, abscess of the lung, and fresh pleurisy, with wide distribution of *Staphylococcus aureus;* nevertheless, the operation wound had remained perfectly healthy without a trace of suppuration.

Into the burning question of auto-infection from the genital tract in puerperal women I cannot enter, save to say that the pyogenic cocci seem to be present only exceptionally in the normal tract and do not thrive there if intentionally put in, but that they are found in pathological uterovaginal secretions, when, of course, they may become a source of puerperal infection. The question is a difficult one and requires further investigation. This passing mention is only intended to indicate that the subject belongs to our theme and may appropriately enter into the discussion.

I must beg permission to defer for a few moments the question of infection from the skin of the operator and of the patient.

That an aseptic wound is not necessarily one free from bacteria has been known since the early days of antiseptic surgery, the subject having been investigated by Ranke, Demarquay, Fischer, Schüller, Watson Cheyne, and others. Kümmell found that pieces of muscle, adipose tissue, or connective tissue taken from fresh wounds immediately after irrigation during the entire operation with corrosive sublimate solution 1 : 10,000, contained bacteria, and the same result was obtained after frequent washing with sublimate solution 1 : 1000.

The most recent investigation of the bacteria in fresh wounds treated antiseptically is by Bossowski, who found, of 50 cases, 10 with negative and 40 with positive result from the bacteriological examination. He found *Staphylococcus albus* 26 times, *Staphylococcus aureus* 9 times, *Streptococcus pyogenes* 2 times, and other organisms, nonpathogenic, 8 times. He several times found a coccus, liquefying gelatin after several days, growing at first white and then slowly turning yellow, and incapable of producing suppuration in rabbits or in wounds. This he proposes to call *Staphylococcus gilvus.*

In every case in which *Staphylococcus aureus* or *Streptococcus pyogenes,* with or without other organisms, was present, suppuration occurred. On the other hand *Staphylococcus albus* produced generally no interference with the healing of the wound, but sometimes it caused a little suppuration of the drain canal or a stitch abscess. In the majority of cases there was absolute *prima intentio.* The small number of the colonies indicated meagre development in the wound.

Drs. Ghriskey and Robb have made under my observation the bacteriological examination of 45 laparotomy wounds treated with strict antiseptic precautions in the gynecological wards of The Johns Hopkins Hospital. The method of treatment and the technique adopted for obtaining the cultures have already been published by Dr. Robb, in the "Bulletin of The Johns Hopkins Hospital," July, 1891.

Of these 45 cases the result was negative in 14, positive in 31, or nearly 69 per cent of the cases. The organisms found were *Staphylococcus albus* 19 times, *Staphylococcus aureus* 5 times, *Bacillus coli communis* 6 times, *Streptococcus pyogenes* 3 times, once alone and twice in combination with the albus. Of the cases with the aureus in only two was there reason to suspect infection from without; in the others this organism was present in the seat of disease for which the operation was performed. In the first case with *Streptococcus pyogenes* the operation was performed for an ovarian abscess which contained streptococci in large numbers; the other two cases probably became infected in some way from the first patient.

In all of the cases presenting *Staphylococcus aureus* or *Streptococcus pyogenes* the wound suppurated, and, as a rule, the general condition of the patient was bad.

In many of the cases where the white staphylococcus was found there was no disturbance in the healing of the wound. This was true especially when the organism was found in small number, and when it made its first appearance subsequent to the first dressing, which took place usually twenty-four to forty-eight hours after the operation. In many cases, however, the white coccus was the cause of more or less trouble, although rarely of a serious nature. It was found to travel down to the bottom of the wound along the side of the drainage tube, and any purulent discharge attributable to its presence was usually confined to the tract occupied by the drainage tube, so that the drainage tube distinctly favored the invasion and growth of the coccus. Sometimes fever without suppuration seemed attributable to the presence of this coccus. That this organism is the most frequent cause of the ordinary stitch abscesses has already been mentioned.

Under especially favorable circumstances this white staphylococcus may cause peritonitis, as is shown by a fatal case of hysteromyomectomy where at the autopsy a volvulus of the ileum was found. The stump and the laparotomy wound both looked healthy, but there was a fresh fibrinopurulent peritonitis clearly starting from that part of the peritoneum agglutinated to the inner edge of the laparotomy wound. This part corresponded to the peritoneum covering the twisted ileum. The twist was not so tight as to have produced gangrene or even a marked haemorrhagic condition; but it had interfered sufficiently with the circulation and the nutrition of the peritoneum to have rendered this a favorable soil for the growth of an otherwise comparatively innocent bacterium, our white staphylococcus having been found in pure culture in the inflamed peritoneum.

The efforts to find out the origin of this very common inhabitant of wounds, treated aseptically or antiseptically, have led us to some interesting and new observations concerning the bacteria of the skin.

The skin may have all sorts of bacteria upon its surface, but, like the mouth and the intestine, in addition to these it has its own distinctive bacterial flora.

If the hands be thoroughly scrubbed with soap and hot water with a sterilized brush, or if this be followed by washing the hands in sublimate solution and the mercury be precipitated by sulphide of ammonium, the cultures obtained from scrapings of the skin so treated will generally be found to contain, as the prevailing organism, the white staphylococcus, and often this will appear in nearly or quite pure culture.

But the most important point is that this coccus is very often present in parts of the skin deeper than can be reached by any known means of cutaneous disinfection save the application of heat. We were directed to this conclusion first by experiments on animals. Then the observation of the same white coccus in pure culture time after time in wounds where every possible antiseptic precaution had been taken, pointed to the same deduction. More conclusive evidence was afforded by the examination of skin stitches in cases where at the time of the operation it was proven that the silk used for stitches was sterile and that the surface of the skin after thorough disinfection was sterile. The silk sutures when removed were proven both by microscopical examination and by roll or plate cultures to contain with great regularity the white staphylococcus, often in considerable number, often enclosed within leucocytes, and this not only where a stitch abscess had formed but also where there was not a trace of suppuration or visible reaction around the stitch.

A crucial experiment is the following: The skin is thoroughly disinfected, in the manner to be presently described, so that culture tubes of nutrient agar or gelatin inoculated by scrapings from its surface remain sterile. A silk thread, sterilized by steam and proven by culture methods to be sterile, is passed one or more times by means of a sterilized instrument through the skin, is withdrawn, and at once a tube of melted nutrient agar is inoculated with the thread and rolled. This amounts to a ready means of making cultures directly from the deeper layers of the epidermis and the skin, and is a method applicable for many purposes to the bacteriological examination of the skin. By this method the presence of the white staphylococcus, often in pure culture, has been repeatedly demonstrated in parts of the epidermis deeper than were acted upon by any methods of disinfection of the surface of the skin. So far as our observations extend, and already they amount to a large number, this coccus may be regarded as a nearly, if not quite, constant inhabitant of the epidermis. It is now clear why I have proposed to call it *Staphylococcus epidermidis albus.* It possesses such feeble pyogenic capacity, as is shown by its behavior in wounds as well as by experiments on rabbits, that the designation *Staphylococcus pyogenes albus* does not seem appropriate. Still, I am not inclined to insist too much upon this point, as possibly this coccus, which has hitherto been unquestionably identified by Bossowski and others with the ordinary *Staphylococcus pyogenes albus* of Rosenbach, is an attenuated or modified form of the latter organism, although, as already mentioned, it presents some points of difference from the classical description of the white pyogenic coccus.

We can now understand how, without any flaw in the antiseptic technique of the surgeon, this microorganism may be present in wounds, and we have a satisfactory explanation of the frequent occurrence of stitch abscesses, although, of course, the inference should not be drawn that the white staphylococcus is the only bacterium which may be concerned in the production of these annoying complications.

How much practical importance attaches to the demonstration of this coccus in the deeper layers of the epidermis or the glandular appendages of the skin I am not prepared to say. The surgeon with good technique who does not bother himself about it is not likely to be severely punished by the behavior of his wounds. Those who put drainage tubes and other extraneous substances into their wounds I think will have to consider it. Dr. Halsted, on the basis of researches on the bacteria of the skin and the difficulties of complete disinfection of the skin of the patient, has abandoned for nearly all wounds the use of skin stitches, the edges of the wound being brought together with admirable coaptation by subcutaneous sutures. The results, both as regards the scar and the aseptic healing of the wound, have been most gratifying. Stitch abscesses are, of course, avoided by this procedure.

Another coccus which we have found, although less frequently than the white coccus, on the surface and in the deeper layers of the epidermis, is one already referred to which corresponds to that called by Bossowski *Staphylococcus gilvus.* This organism seems to bear about the same relation to the pyogenic yellow staphylococcus which our epidermal white coccus does to the typical pyogenic white staphylococcus.

Staphylococcus pyogenes aureus we have found very frequently on the hands of surgeons and their assistants who have to do with suppurating cases of any kind. It may be present in this situation at least for several days after contact with surgical cases. It was met only exceptionally upon the hands of other persons.

The demonstration of microorganisms in layers of the skin deeper than can be disinfected by present methods suggests that more attention than seems now to be customary should be paid to the skin of the patient as a source of traumatic infections. It also admonishes us to receive with caution the statements recently made concerning the elimination in suppurative diseases of staphylococci by the sweat.

A considerable part of our work has been devoted to the subject of surgical antisepsis, and particularly to the disinfection of the skin. Dr. Abbott has conducted in my laboratory careful experiments regarding corrosive sublimate as a disinfectant against *Staphylococcus pyogenes aureus,* in which many fallacies in previous work on the same subject have been pointed out. In view of the time already consumed, I must ask permission simply to refer to Dr. Abbott's paper on this subject, already published in the "Bulletin of The Johns Hopkins Hospital" for April, 1891.

The conditions for the efficient action of chemical disinfectants have been found to be far more complicated and less easily controlled than was formerly supposed, and the substitution, wherever applicable, of the simple and certain methods of disinfection by heat, such as have been long employed in bacteriological laboratories, is to be commended. Chemical disinfectants still have their place for many purposes in the operating room, but their place is not in fresh, healthy wounds.

Thorough scrubbing of the skin with soap and warm water by a sterilized brush removes many bacteria, but not all, and it cannot be regarded as a satisfactory means of cutaneous disinfection.

The fallacy in previous work on disinfection of the skin with corrosive sublimate has been that in testing its efficiency the sublimate was not first precipitated by sulphide of ammonium or some other alkaline sulphide. If this precaution, to which attention was first directed by Geppert, be observed, it will be found that corrosive sublimate accomplishes much less than is generally supposed. Our revision of the work relating to cutaneous disinfection with sublimate has led to some curious and interesting observations, and to results which at first seemed paradoxical.

By examining the hands of surgeons who are in the habit of washing them daily in solutions of corrosive sublimate, it was found that the mercury becomes so intimately incorporated with the epidermis that its presence there can be demonstrated by means of sulphide of ammonium at least many days after any contact with mercurial solutions has taken place. Micrococci in the epidermis which have not been killed by washing in sublimate solutions, but which have been brought into such relation to the sublimate that even after prolonged washing of the skin with alcohol and water they will not grow on culture media until the skin is washed with sulphide of ammonium, may also remain a long time in the epidermis. Hence it may happen that prolonged scrubbing of the hands of such persons simply with soap and warm water may remove so many superficial bacteria that the cultures from the scrapings of the epidermis may show very few, sometimes even no, colonies; whereas, when this is followed by washing in sublimate and then in sulphide of ammonium, a much larger number of colonies appear in the cultures. This apparently paradoxical result, which is obtained only from the hands of those who have previously washed them in sublimate solution, has no reference to the application of the sublimate immediately after the soap and water, but is to be explained by the liberation, by means of sulphide of ammonium, of the bacteria held in check by the mercury used, it may be, several days before the experiment. The same result is, of course, obtained if the sulphide of ammonium be applied immediately after the scrubbing with soap and water. These observations upon the persistence of mercury in the epidermis and its long continued inhibition of the growth of bacteria, make it necessary in all work upon disinfection of the hands to first precipitate the mercury with sulphide of ammonium whenever the experiments are to be made upon hands which have been washed in sublimate solutions, even if this has occurred a long time previously. Exactly what relation the mercury in the epidermis holds to the bacteria which it does not destroy, but whose growth in our nutrient media it prevents, we cannot say. We may, perhaps, think of these bacteria as enveloped in an albuminous combination of mercury. One thing is certain—that, when the sublimate has been as thoroughly washed off from the skin as possible with water, or has been applied days before, the nutrient gelatin or agar is not rendered unfit for the growth of bacteria by the mere presence of the small quantity of mercury carried into it with scrapings from the epidermis, for the bacteria which have reached the epidermis after the application of the sublimate—and these are often identical with those inhibited by the mercury—develop as usual. It is only those bacteria which were originally brought into contact with the sublimate in some such manner as that suggested which will not grow until after the application of sulphide of ammonium, and it is not—as has been usually supposed in other observations of a similar kind in disinfectant experiments with sublimate—the alteration of the nutrient medium by the presence of a trace of sublimate which inhibits the growth of the colonies.

As to the practical efficiency of disinfection of the skin with solutions of corrosive sublimate, it is to be said that this agent, when properly applied, kills most of the bacteria upon the surface of the skin. The washing of the skin with alcohol immediately before the use of the sublimate increases its efficiency to a marked degree. If Fürbringer's method be carried out according to the strict letter of his directions it yields fair results, but it is not certain. If the mercury after employment of this method be precipitated by washing the hands in sulphide of ammonium, it will be found that the results are much less favorable than would appear by cultures made from the skin and under the nails, without the use of ammonium sulphide. It is especially the scrapings under the nails and around the matrix of the nails which yield positive results when ammonium sulphide is used, but often negative ones without this precaution. It need hardly be said that in our experiments all of the well known, although often neglected, precautions to insure the full strength of the sublimate solutions were observed.

It may be urged that it is not necessary actually to kill the bacteria upon the skin; it is sufficient if they are rendered incapable of growth, and as most of those which are not killed by the sublimate do not grow upon our ordinary nutrient media, it is reasonable to infer that they will not grow in wounds. This line of argument certainly deserves consideration; nevertheless, there is no positive proof that these bacteria will not grow in wounds under some conditions, and surely one will feel safer with a method of disinfection which actually kills the bacteria.

I shall not detain you with the results of our experiments with other disinfectant agents. These will be published in a short time elsewhere. I shall simply state here that we have thus far obtained the best results in disinfection of the skin by the following method:

1. The nails are kept short and clean.
2. The hands are washed thoroughly for several minutes with soap and water, the water being as warm as can be comfortably borne, and being frequently changed. A brush, sterilized by steam, is used. The excess of soap is washed off with water.
3. The hands are immersed for one to two minutes in a warm saturated solution of permanganate of potash and are rubbed over thoroughly with a sterilized swab.

4. They are then placed in a warm saturated solution of oxalic acid, where they remain until complete decolorization of the permanganate occurs.

5. They are then washed off with sterilized salt solution or water.

6. They may then be immersed for two minutes in sublimate solution, 1:500.

The bacteriological examination of the skin thus treated yields almost uniformly negative results, the material for the cultures being taken from underneath and around the nails. This is the procedure now employed in the gynecological and surgical wards of the hospital.

The principal conclusions of this paper may be summarized as follows:

The number of the different species of bacteria, particularly of bacilli, revealed by the systematic study of traumatic infections is much greater than was formerly supposed. The pyogenic staphylococci and streptococci, however, are by far the most common causes of suppurative affections of wounds.

A coccus, which may appropriately be called *Staphylococcus epidermidis albus,* is a nearly, if not quite, constant inhabitant of the epidermis, lying both superficially and also deeper than can be reached by present methods of disinfection of the skin. This coccus is found frequently in aseptic wounds. It may be the cause of disturbances, usually of a relatively slight degree, in the healing of the wound, especially when drainage tubes are inserted. It is the most common cause of stitch abscesses in wounds treated antiseptically or aseptically.

Bacillus coli communis is a frequent invader of various organs of the body in cases with ulcerative or other lesions of the intestinal mucous membrane. In such cases its presence is usually unattended by the evidence of pathogenic action, but this bacillus may be associated with inflammatory affections of wounds, with peritonitis, and with abscesses.

There are many reasons for believing the process of suppuration serves a useful purpose in combating bacteria and preventing their invasion of the circulating fluids and the tissues of the body.

The pyogenic bacteria set up suppuration by means of chemical substances produced by them and entering into their composition. The studies of chemotaxis have shed much light upon the mode of action of these substances.

The effects produced in the animal body by the pyogenic cocci are determined by many factors relating to the infectious agents and to the individual exposed to infection. There are differences in these effects depending upon the species of animal, upon the tissues and part of the body infected, upon the readiness of absorption from the infected part; upon the source, the number, and the virulence of the organisms; upon the nature and amount of the toxic substances accompanying and produced by the bacteria, upon general predisposing conditions of the body, and upon local conditions in a wound such as the presence of foreign bodies, of pathological products, of dead spaces, of bruised, necrotic, and strangulated tissues.

Infectious agents, as they occur under natural conditions, may possess greater virulence than the same bacteria in artificial cultures, and this probably depends upon accompanying toxic substances.

Results of experiments on animals explain clinical experience concerning the aseptic healing of wounds by the so-called organization of a blood-clot.

The tissues of a wound should be handled so as to interfere as little as possible with their vital capacity to overcome bacteria.

Although the greatest danger of infection of a wound from without is by direct contact, nevertheless the possibility of infection from the air should not be disregarded.

Auto-infection may take place by the entrance into the circulation and tissues of pyogenic bacteria from the alimentary and the genital canals, but there is no evidence that this can occur when these tracts are in a healthy condition. Moreover, with the requisite lesions of these tracts other general and local conditions of the body are important, if not essential, factors in bringing about pyogenic or septic infection.

The presence in the circulating blood and tissues of certain chemical products of pyogenic and of putrefactive bacteria, as well as that of various other injurious substances, favors the growth in wounds of septic and pyogenic bacteria, both of those which may be carried to the part by the circulating fluids and those which may enter from outside of the body.

Whenever we have been able to demonstrate the presence in wounds in human beings of *Staphylococcus pyogenes aureus* or of *Streptococcus pyogenes* the wound either was suppurating or subsequently it suppurated.

Only in the minority of cases were the aseptic wounds which we examined free from bacteria. By far the most common organism in these wounds pursuing an aseptic course is *Staphylococcus epidermidis albus,* which without the presence of a drainage tube or other foreign body rarely causes suppuration in the wound.

The presence of microorganisms in layers of the skin deeper than can be reached by existing methods of cutaneous disinfection points to the skin, especially to that of the patient, as a source of infection to be carefully guarded against.

The substitution so far as possible of subcutaneous for cutaneous sutures lessens the chances of infection from this source, and particularly those of stitch abscesses.

Wherever applicable in surgical antiseptics, disinfection by heat should be preferred to that by chemical agents.

Previous experiments to determine the efficacy of disinfection of the skin with corrosive sublimate are vitiated to a considerable extent by the failure to precipitate the mercury in the form of the sulphide before testing by culture methods its germicidal power on the skin.

The mercury may remain for days and weeks intimately incorporated with the epidermis.

Epidermal bacteria not killed by the sublimate may be brought into such relation with it that they will not grow in ordinary culture media until the mercury is precipitated as the sulphide, and such bacteria may remain for days and weeks in the epidermis.

The results of Fürbringer's method of disinfection of the skin are found to be less favorable when they are tested after precipitation of the mercury with ammonium sulphide than without this precaution.

The best results in cutaneous disinfection we obtained by a method in which permanganate of potash followed by oxalic acid plays the principal disinfectant rôle.[2]

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[2] Cultures were exhibited showing various species of bacteria mentioned in the paper, the white staphylococci and other bacteria isolated from the epidermis, the growths from skin sutures, and illustrating the results of different methods of disinfection of the skin, and the importance of precipitation of the mercury with ammonium sulphide when testing the germicide action of corrosive sublimate to the skin.

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SOME CONSIDERATIONS CONCERNING ANTISEPTIC SURGERY[1]

The greatest achievement of modern surgery is not so much the triumph of abdominal or of cerebral surgery, or of any particular class of operations, as it is the method which has brought into the surgeon's domain every organ of the human body. The victories of this method are manifested not less in the aseptic healing of the wound resulting from the removal of a simple wen, than in the recovery without suppuration and fever after the extirpation of a cerebral tumor, or of a large abdominal tumor.

Modern surgery begins with the introduction of antiseptic methods of wound treatment, and it is the imperishable merit of Lister to have ushered in this era by his recognition of the great truth that the infection of wounds depends upon the presence of bacteria and by his adoption of measure of treatment based upon this truth. Upon the establishment of these fundamental principles his fame will rest, even if every detail in his original method of treatment be replaced by means found better suited to the purpose.

No apology is needed if a pathologist ventures to speak to you about a subject with such practical bearings as surgical antisepsis, when it is recalled that antiseptic, and aseptic methods of wound treatment rest upon bacteriological work no less than upon clinical experience. In the development of these methods the surgeon and the bacteriologist have worked hand in hand. The harmony and cooperation between the worker in the laboratory and the practical physician and surgeon have never been so great as at the present time. On the one hand, the experimenter applies himself to problems of immediate interest to the practitioner and seeks the aid of clinical observation as never before, and the practitioner is eager to learn and to apply the established results of experiment. That this combined work is calculated to advance scientific and practical medicine in the surest way cannot be doubted.

Lister's original antiseptic methods, which were introduced about the middle of the sixth decade of this century, were founded mainly upon the views which prevailed at that time as to the relation of bacteria to the processes of putrefaction and fermentation. One chief source of the bacteria which infect wounds was regarded as the air; and by the use of carbolic acid spray he hoped to disinfect the air over the field of operation. Lister also employed carbolic acid as the agent for disinfecting the wound, hands, instruments and all that might come in contact with the wound. The dressings applied to the wound were permeated with the disinfectant and were expensive and somewhat complicated. The method necessitated extensive use of drainage tubes and frequent change of dressings.

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[1] Address delivered before the Clinical Society of Maryland, October 16, 1891. Maryland M. J., Balt., 1891-2, XXVI, 45-57.

The results of this method marked a great advance in surgery, the contrast between the new and the old method being more evident in Germany than in Great Britain, because in the former the hospitals were, in general, older, less cleanly and in a less sanitary condition. Good as were the results of the new method, they were still so far from perfect that efforts continued to be made to improve the method.

It is evidence of the value and fructifying influence of the new ideas introduced by Lister that they have proven capable of wide development; that they have stimulated workers in science and in practical medicine to efforts which have extended our knowledge far beyond the mark of a quarter of a century ago; and that they have occasioned unprecedented activity and progress in surgery during this period. Permit me to direct your attention to some of these advances in the general principles of antiseptic surgery, looking at the subject rather from the point of view of the bacteriologist than from that of the practical surgeon. In the limits of a paper of this kind it is possible to select for consideration only a few points, leaving many of great importance untouched.

The most fundamental advance relates to the extension of our knowledge concerning the nature and habits of the microorganisms which cause traumatic infections. As has already been said, Lister's early work was based upon analogies drawn from the relation of bacteria to the processes of fermentation and putrefaction. Nothing or almost nothing was known as to the bacteria actually concerned in suppuration and other wound infections. The observations of Klebs, Recklinghausen and others at this period concerning the presence of micrococci in pyaemia and other suppurative conditions gave little information as to the character and behavior of these organisms.

The new era in bacteriology was introduced by the publication of Robert Koch's "Untersuchungen über die Aetiologie der Wundinfectionskrankheiten," in 1878. The kind of evidence necessary to prove the causation of a disease by a specific microorganism was here clearly set forth and was brilliantly illustrated by his study of a number of new infectious diseases, chiefly experimental septicaemias, produced in mice and rabbits by the inoculation of foul substances. Three years later, Koch gave to the world the details of his method of cultivating bacteria upon solid transparent media, without which most of the great discoveries in bacteriology during the last decade could not have been made.

Although Koch's epoch-making publication was entitled "Investigations Concerning the Etiology of Traumatic Infections," this did not relate to the bacteria which cause wound infections in human beings; and it was hardly less misleading when inferences were drawn as to the nature and mode of causation of suppurative and other traumatic infections of human beings from supposed analogies with Koch's experimental septicaemias, than from those with fermentation and putrefaction.

The first thorough study by modern bacteriological methods of the bacteria causing suppuration in human beings was made by Ogston, Rosenbach and Passet, between the years 1880 and 1885. To this period belongs also the study and first cultivation of the erysipelas coccus by Fehleisen. Ogston confined his attention to the microscopical observation, by the improved optical aids, of the staphylococci and streptococci of suppuration. The other investigators isolated in pure culture and studied the different species of bacteria found in abscesses. These fundamental researches made us familiar with the ordinary pyogenic staphylococci and streptococci. Now, for the first time, the causation of the most common, and, therefore, the most important, of all traumatic infections, namely, the suppurative, could be satisfactorily studied. The commonest enemies of the surgeon were known, their distribution in nature, the ways in which they may gain entrance to wounds, their habits and behavior both inside and outside of the body, were open to direct observation and experiment. It could not be doubted that this increased knowledge would lead to more efficient means of combating the enemy, and this has proven true.

Although we have learned that many other species of microorganisms than those originally described by Ogston, Rosenbach and Passet may be concerned in traumatic infections of human beings, nevertheless the pyogenic cocci are by far the most common and important, and these are the ones which have been most thoroughly studied. It soon became evident that the study of infection with the pyogenic cocci presents many peculiar and complicated problems; and we owe especially to Grawitz and his pupils the demonstration that under ordinary conditions, the healthy tissues in many situations are capable of disposing of a large number of these cocci without manifest injury, and also the recognition of several abnormal conditions which deprive the tissues of this power.

It is becoming every day clearer that the most valuable friend which the surgeon has in his efforts to secure aseptic healing of his wounds is the capacity of the fluids and cells of the animal body to overcome invading microorganisms. The indication is no less urgent than ever before to keep bacteria out of wounds in every way possible, but it seems plain that hitherto the efforts of the antiseptic surgeon have been directed too exclusively toward this indication and toward the attempted destruction of bacteria which may have accidentally entered the wound, and not enough toward preserving, as far as possible, the germicidal power of the tissues and fluids.

Among the influences which have been found favorable to the growth in wounds of bacteria, which otherwise might be disposed of by the tissues and animal fluids without harm, are the presence of foreign bodies, such as drainage tubes and coarse ligatures, the necrosis and degeneration of tissue caused by the contact of strong chemical disinfectants with the wounded tissues, the strangulation of masses of tissue with ligatures, the strangulation, tension and interference with the circulation often caused by deep coaptation sutures, and interference with the circulation and vitality of tissues which may result from too forcible pressure. If it were within the power of the surgeon to keep bacteria entirely out of wounds, or to destroy them without damage to the tissues after they enter, the conditions just mentioned could not by themselves alone cause infection, but this power he does not at present possess, as is demonstrated by the bacteriological examination of wounds treated aseptically or antiseptically. Hence it behooves him to learn all that he can concerning those conditions which favor and those which are hostile to the development of bacteria in wounds, and to endeavor to retain and produce the hostile and to remove the favorable conditions for the growth of bacteria.

That conditions predisposing to suppurative and septic complications of wounds are to be sought not only in local conditions about the wound, but also in the general state of the patient, is known to every surgeon of experience, and this latter side of the subject has been recently presented in an interesting and suggestive manner by Dr. Roswell Park, in his paper before the Congress of American Physicians and Surgeons.

It has been a stumbling block in the way of the acceptance of the general principles of antiseptic surgery by some, that surgeons who claim to disregard these principles have good results even in operations which were considered unwarrantable before the introduction of antiseptic methods. The foremost champion of these dissenters is, perhaps, Lawson Tait, so widely known as a successful laparotomist. But there is no difficulty in explaining these results, which in reality furnish no sound argument against the employment of proper antiseptic measures in the treatment of wounds. Mr. Tait and his followers make extensive, although not sufficient, use of the most efficient of all antiseptic agents, namely, heat. By attention to cleanliness they greatly diminish the chances of infection of wounds by bacteria.

They are often particularly rapid and skillful operators. Above all they respect the vitality of the tissues, especially by withholding the application to them of chemical disinfectants. They do not disregard the principles of antiseptic surgery to the extent that they may suppose they do, but they would be wiser if they paid still more attention to these principles. Because the use of unsterilized ligatures or the washing of the peritoneal cavity with unsterilized water is not attended by bad results in many cases, is no proof that it is not safer to sterilize ligatures and all that comes into contact with a wound.

Moreover, there are special reasons, now well known, why the results of operations in the peritoneal cavity cannot be regarded as the best test of antiseptic methods. The healthy peritoneum is tolerant to an extraordinary degree of the presence of pyogenic bacteria, but when these conditions of tolerance are overstepped then the dangers from infection surpass those from infections of wounds of external parts. These exceptional dangers are what render the employment of proper antiseptic measures especially imperative in laparotomies.

The tendency at the present time to abandon the application of strong disinfectant solutions to fresh wounds and to pay more attention to the gentle handling of wounded tissues has been interpreted by some as a reaction against antiseptic surgery and a return to ideas which obtained in preantiseptic days. It is true that the capacity of the living tissues to resist putrefaction and the influence upon the process of healing of the character and condition of wounds are very old conceptions in surgery. The old books on surgery have much to say on these points, and entered into extreme refinements in the classification and description of wounds, with especial reference to their behavior in the process of repair. They contained much sound doctrine in these matters, which can still be perused with profit. But the results of the old and those of the new surgery are alone sufficient to prove the wide difference between them. Under the old régime the healing of wounds without suppuration was so exceptional that the occurrence could even be disputed by prominent surgeons, whereas now the surgeon with good technique may look with reasonable certainty to this result, once so exceptional. The foundations of antiseptic surgery are strengthened, not shaken, by improvements in details and by the recognition of new principles. Now that we recognize the dangers of applying strong antiseptic substances to wounds and the difficulties of disinfecting wounds in this way, the arguments are stronger than ever before for operating with as nearly sterile surroundings as possible and for keeping the wound from contact with unsterilized objects.

Permit me to say in this connection that I fail to see the advantage of using the term aseptic surgery in the sense in which it is generally employed at the present time as something distinct from antiseptic surgery. One hears on all sides such expressions as that aseptic surgery is displacing or is destined to displace antiseptic surgery. What is understood by aseptic surgery undoubtedly marks an important advance in surgical antisepsis, but it is none the less antiseptic surgery. It would seem as if some supposed that antiseptic surgery means nothing more than the use of chemical disinfectants in the management of wounds, or that antiseptic technique is incapable of improvement. An antiseptic agent is anything which has the power of arresting the development of septic bacteria. With his views as to the influence of blood in a wound in favoring infection, von Bergmann has said that a ligature is one of our most important antiseptic agents. The substitution of heat for chemical substances in the disinfection of instruments, ligatures, dressings, etc., is an important advance but it is only the substitution of a more powerful for a weaker antiseptic agent. The object of antiseptic surgery is to secure an aseptic condition of a wound, and here it may be well to remark that this rarely is equivalent to the condition generally understood in a bacteriological laboratory as aseptic, namely, freedom from bacteria.

The abandonment of disinfectant applications to a wound is for the purpose of securing the action of more efficient antiseptic agencies under the circumstances, namely, the fluids and cells of the surrounding tissues, and this abandonment renders all the more urgent the use of antiseptic agents outside of the wound. It is doubtless one of the most useless occupations to contend against current usage in medical nomenclature, but it seems to me unfortunate that the term aseptic surgery should be introduced as if it signified something different from antiseptic surgery and not simply an improvement in the methods of antiseptic surgery.

It is interesting from a bacteriological point of view to note the improvement in the dressing and management of wounds since the pioneer work of Lister. The early efforts in this direction, especially in Germany, were to render the dressings less expensive and less cumbersome, items of no small consideration in a hospital with limited resources. Important advances in principle, however, resulted from the efforts to do away with the frequent changing of surgical dressings, which was necessitated by Lister's early method. The employment of absorbent material for the dressings without the outside impermeable covering adopted by Lister, permits rapid drying of the secretions and, therefore, rests upon the sound basis that bacteria find in moist media opportunities for their growth not furnished by dry material. Every bacteriologist knows that his culture tubes are much more likely to become contaminated if he places a rubber cup over the cotton wool at the mouth of the tube than without this precaution, unless he takes especial pains to sterilize the cotton wool and the mouth of the tube before applying the rubber. It is almost impossible for the surgeon to keep his dressings for any length of time free from bacteria, and it is, therefore, advisable not to shut these bacteria into secretions and an atmosphere saturated with moisture, prevented, by an impermeable covering, from evaporation.

Some of the most important advances in antiseptic surgery have come from the efforts to dispense with the use of drainage tubes. The drainage tube question is a part of the much larger one: How shall the so-called dead spaces, that is, the cavities and recesses, in a wound be managed? The surgeon has at his disposal the choice between many various means for managing these spaces. He can drain the spaces, or stuff them with foreign material, or endeavor to obliterate them by pressure, or by buried sutures, or by inverting sutures, or by transplantation of flaps, or he can permit them to fill with blood, or he can let them alone. Doubtless no one of these methods is universally applicable; probably each one has its field of usefulness. The skill of the surgeon consists in large measure in the manner in which he exercises his judgment as to the management of the dead spaces in a wound, and I shall venture to say a few words on this important but still unsettled question only from a bacteriological point of view.

The following objections to the insertion of drainage tubes into wounds suggest themselves: *First.* They tend to remove bacteria which may get into a wound from the bactericidal influence of the tissues and animal juices. *Second.* Bacteria may travel by continuous growth or in other ways down the sides of a drainage tube and so penetrate into a wound which they otherwise would not enter. We have repeatedly been able to demonstrate this mode of entrance into a wound of the white staphylococcus found so commonly in the epidermis. The danger of leaving any part of a drainage tube exposed to the air is too evident to require mention. *Third.* The changing of dressing necessitated by the presence of drainage tubes increases in proportion to its frequency the chances of accidental infection. *Fourth.* The drainage tube keeps asunder tissues which might otherwise immediately unite. *Fifth.* Its presence as a foreign body is an irritant and increases exudation. *Sixth.* The withdrawal of tubes left any considerable time in wounds breaks up forming granulations and thus both prolongs the process of repair and opens the way for infection. Granulation tissue is an obstacle to the invasion of pathogenic bacteria from the surface, as has been proven by experiment. *Seventh.* After removal of the tube there is left a tract prone to suppurate and often slow in healing.

The advantage from the employment of drainage tubes is, of course, the removal of secretions, and this indication becomes an urgent one if the cavity with which the tube communicates becomes infected and suppurates. In a given case the surgeon must weigh the advantages and the disadvantages and act according to his judgment. The practice of many surgeons at the present time, of restricting within much narrower limits than formerly the use of drainage tubes and of discarding them for all wounds which offer a fair prospect of primary union, is a distinct advance in the technique of antiseptic surgery.

The practice of stuffing cavities with foreign substances, even those of an absorbable nature, is open to most of the objections already urged against drainage tubes.

Much can be accomplished by external pressure in bringing together the surfaces within a wound, but this procedure is of only limited application, for on the one hand only such spaces can be obliterated in this way as are favorably situated and have surfaces suitable for coaptation, and on the other hand there is difficulty in regulating the pressure so that it does not interfere with the circulation and with the vitality of the tissues.

The method elaborated especially by Neuber and by Küster, of obliterating spaces in a wound by means of deep coaptation sutures, has unquestionably given excellent results, but it is not free from serious objections. The conscientious execution of the method often is tedious and greatly prolongs the operation, and it may be doubted whether the object aimed at is not often missed. The most manifest objections however, are the presence in the wound of so many foreign bodies in the form of sutures as are often required, and especially the danger of strangulating and stretching unduly the tissues, and thus interfering with their antibacterial capacities. The thinner the sutures employed, the smaller is the danger of their presence as foreign bodies.

The idea that blood in a wound is a dangerous thing is a prevalent one, and such surgeons as Küster and von Bergmann in recent years have dwelt especially upon this danger. Blood is looked upon as a foreign substance more dangerous than most foreign substances because it is held to be especially prone to decomposition by bacteria. Nevertheless, John Hunter was fond of dwelling upon what he called the vitality of blood, and was acquainted with its so-called organization in wounds. Mr. John Chiene, about fifteen years ago, directed attention anew to the same views; but it was Schede who in 1886, in an article on the "Healing of Wounds Under the Moist Blood Scab," brought prominently to the notice of surgeons the value of the blood-clot in the healing of a certain class of wounds. The discoveries more recently made as to the power of fresh blood outside of the body to destroy many kinds of bacteria (although in only very limited degree the pyogenic cocci) or their poisonous products have shed additional light upon this subject. The credit of introducing as a method of general applicability the management of dead spaces by permitting them to fill with blood belongs to Halsted. Before him several surgeons, and Schede especially, had recommended the use of the blood-clot for wounds, but Halsted has substituted for all other methods previously employed in treating dead spaces not otherwise easily obliterated, that is, for drainage tubes, buried sutures, pressure, etc., the use of the blood-clot as the method of widest applicability and as affording the best results. In a paper read before the recent Congress of American Physicians and Surgeons, I described the results of my experiments on animals, which supported the conclusions derived from clinical experience as to the value of the method of healing of wounds by allowing spaces to fill with blood. That the unintended presence of blood in a wound may be a source of danger by occasioning undue tension and in other ways, and is a very different thing from the purposeful application of the method of healing by so-called organization of blood-coagula, hardly needs to be mentioned. Nor need I emphasize the fact that loss of blood, as such, is to be guarded against, for it is proven experimentally that general anaemia is a predisposing cause of infection with pyogenic bacteria.

The question suggests itself, What would happen under good antiseptic technique if the so-called dead spaces of a fresh operation wound were left to themselves, that is, were neither drained nor obliterated nor permitted to fill with blood? There is not sufficient experience to answer this question satisfactorily. This, however, is to be considered. Exudations composed of fluid and cells would quickly accumulate in most of these spaces, and these fluids and cells possess antibacterial properties as well as blood. Granulation tissue would spring up from the sides. I venture to predict that if the surgeon paid no particular attention to the filling or obliteration of many of the smaller spaces in fresh operation wounds, and if the antiseptic technique were good, the process of healing would be as satisfactory as by any of the other methods mentioned.

Let us now turn our attention to the progress which has been made in the employment of antiseptic agents for surgical purposes. Soon after the introduction of Lister's methods, the search began for new and better disinfectants than carbolic acid. This search brought to light a large number of agents, some good, but more of little or no value for the purpose intended, and in general, carbolic acid continued to possess the field until Koch, by his fundamental experiments with disinfectants, demonstrated the preeminent germicidal power of corrosive sublimate. This latter disinfectant has, to a large extent, although not entirely, taken the place formerly occupied by carbolic acid as the surgical disinfectant *par excellence.* In no department of antiseptic surgical technique has the value of bacteriological methods of experimentation been more evident than in establishing the germicidal powers of various real or supposed disinfectants; nevertheless. the importance of not rejecting sound clinical experience in this matter is shown by the fate of iodoform as an antiseptic agent. Iodoform would probably never have been recommended as an antiseptic on the basis of bacteriological experiments alone. Still, the benefits of its employment in wounds and surgical affections are undoubted, and a more careful study of the mode of action and the conditions required for the development of the antiseptic powers of iodoform has justified the use of this substance. A class of disinfectants obtained by fractional distillation at high temperatures of coal-tar oils has recently been introduced, and promises valuable service. These are the higher cresols, which pass over by distillation between 190° and 210° C. The difficulty has been to obtain these oils in a form soluble in water. Creolin is a fine emulsion of these cresols; and lysol, a solution of the same in water by a soap combination. Although, like most new disinfectants, creolin and lysol have not sustained the extravagant claims made for them by their early enthusiastic advocates, nevertheless they possess strongly disinfectant and other valuable properties and are a useful contribution to the list of surgical antiseptic agents.

But surgical disinfection has progressed not so much by the introduction of new chemical disinfectants as by a better understanding of the mode and conditions of their efficient action. It was once supposed that the skin, wounds, instruments, sponges, etc., could be completely disinfected, without any especial precautions, by being placed for a short time in the disinfectant solutions. Misleading inferences were drawn from the powerful germicidal action of chemical disinfectants when brought into contact with bacteria under the most favorable conditions, namely, with bacteria on silk threads or suspended in water. Not sufficient attention was paid to one of the most important results of Koch's investigations of disinfectants—that the presence of oil or fat prevents the parasiticide action of chemical disinfectants. From this it should have been clear that the presence of oily substances in the epidermis, and often, also, on instruments, sponges and other objects used by the surgeon, must frequently render his efforts at disinfection nugatory, unless by the use of alcohol or by thorough mechanical scrubbing with soap and water, he first removes the grease. Hence it might easily happen that those who scouted the use of chemical disinfectants but employed thorough mechanical cleansing of hands, instruments, etc., really attained a higher degree of antisepsis than those who trusted exclusively to chemical disinfection. Thorough mechanical cleansing and scrubbing with soap and water, although it accomplishes much, does not generally bring about complete disinfection and cannot, therefore, alone be trusted, but these measures are essential adjuvants of any method of surgical disinfection with chemical agents. It may be mentioned that the alkaline soaps possess some disinfectant power, often quite as much as especially prepared commercial disinfectant soaps.

The differences in germicidal power of corrosive sublimate under different conditions are much greater than those of carbolic acid and most other disinfectants. Aside from the presence of oily material, the great obstacle against the disinfectant action of corrosive sublimate in the animal tissues and fluids is its precipitation with albuminous substances. So great is the obstacle that it may be doubted whether it is possible to disinfect by means of corrosive sublimate wounds infected with bacteria, and whether irrigation with solutions of corrosive sublimate accomplishes much more in this way than that with sterile salt solution. We owe especially to Geppert the demonstration that former methods of testing the germicidal power of corrosive sublimate were subject to such grave errors that their results cannot be trusted. Geppert has shown that it is necessary to get rid of the sublimate, which can best be done by precipitation with sulphide of ammonium (or other alkaline sulphides), in order to determine whether bacteria subjected to its action have actually been killed or not. When this precaution is taken it is found that corrosive sublimate is a far less energetic disinfectant than has been generally supposed. For example, Koch believed from his experiments that corrosive sublimate in the strength of 1-1000 destroys anthrax spores in one minute, whereas Geppert has shown that the same strength of sublimate may not have killed all of the spores in watery suspension even at the end of 72 hours. In a recent article Geppert has shown that it is really very difficult to determine whether all of the spores are killed or not by sublimate, inasmuch as a definite concentration of the solution of sulphide used for precipitation is required to test this point and the failure to obtain cultures or to infect an animal is no proof that the spores have been killed, for a different concentration of the substance used to precipitate the sublimate or the employment for this purpose of some substance less injurious to the spores might demonstrate still greater resistance against sublimate. Dr. Abbott has gone over the question of disinfection of *Staphylococcus pyogenes aureus* with corrosive sublimate in my laboratory and his results in conformity with those of Geppert for other bacteria have been made known to you. It may be that the powerful inhibitory and attenuating action of corrosive sublimate upon bacteria is all that is required for the purposes of the surgeon, but it should be understood that this is not equivalent to disinfection, which means the actual destruction of bacteria.

In view of the difficulties and uncertainties of securing under many conditions complete disinfection with chemical agents, especially with corrosive sublimate, it marks a great progress in surgical antisepsis that heat, in the form of dry heat, or live steam, or boiling, is now used so extensively by the surgeon for sterilization of instruments, ligatures, dressings, etc. That chemical disinfectants still have and are likely to continue to have important uses in surgical practice goes without saying.

As I have recently, before the Congress of American Physicians and Surgeons in Washington, presented some of the results of our experience with methods of disinfection of the skin, I shall utilize the present occasion to demonstrate to you a number of culture tubes which illustrate the results of experiments begun by Dr. Halsted, and carried farther by Drs. Ghriskey and Robb, in my laboratory.

A fact important to know in connection with experiments on cutaneous disinfection is that the mercury, probably in combination with albuminous material, remains for many days in the epidermis of hands which have been frequently washed in solutions of corrosive sublimate in the manner customary with surgeons, and this notwithstanding the ordinary daily ablutions with soap and water. Of this I will ask any surgeon present who uses frequently corrosive sublimate for disinfection of his hands to convince himself by placing his fingers in this solution of ammonium sulphide. A mere brownish discoloration of the surface of the skin after applying ammonium sulphide must not be taken as evidence of the presence of mercury, for this in varying degree can take place with those who have not been in contact with mercurial solutions, but it is the rapid deep brown or black discoloration, especially of the nails, which indicates the formation of sulphide of mercury. A pretty demonstration of the growth of the nails is furnished by the absence of the dark brown color in the part of the nail which has grown since the use of the sublimate solution. Not only may the mercury remain for a long time in the epidermis, but it is capable of preventing the growth of bacteria with which it originally came into contact when scrapings from the epidermis are placed in nutrient agar or gelatine. It does not, however, seem to exert any such inhibitory action upon bacteria which have become attached to the epidermis after the application of the sublimate. That epidermal bacteria which fail to grow in culture media inoculated with scrapings from the surface of the skin after washing with sublimate solutions are not necessarily dead, is proven by their development when the scrapings are taken from the same skin after precipitation of the mercury in the epidermis with sulphide of ammonium. For this result the experiments of Geppert had already prepared us, and it is the failure to precipitate the mercury with an alkaline sulphide which led Fürbringer to apparently better results than we have obtained in testing his method of disinfection of the hands with corrosive sublimate. It is also clear that erroneous conclusions may be derived from testing the germicidal power of any other disinfectant on the skin, if the hands of those who are in the habit of using sublimate solutions on them be selected as the object of disinfection, unless the precaution be taken first to get rid of the sublimate, which is best done by precipitating it as the sulphide of mercury.

If the hands have been washed, say the day before, in a solution of corrosive sublimate, then it may happen that most or all of the bacteria which have become attached to the skin since then can be removed by simple scrubbing with soap and water, or by some solution the disinfectant powers of which it is desired to test, and the cultures made from scrapings of the epidermis prove sterile. That, however, there are still living bacteria in the superficial layers of the epidermis, may be shown by making the cultures after washing the hands in solutions of ammonium sulphide. It is probable, from Geppert's recent work, that we should have obtained even more striking results if we had paid more attention in the experiments to the concentration of the solutions of sulphide of ammonium, but we were not familiar then with the importance of this point, and, as it is, the results were sufficiently striking.

I show you here a series of cultures which are intended to demonstrate the points mentioned. Scrapings from the epidermis or beneath the nails were made with a sterilized knife constructed for the purpose, and were transferred to liquefied agar, which was then rolled or poured into Petri's dishes.

In this first series of agar roll cultures there are three sets of tubes which have been inoculated with scrapings from the epidermis and beneath the nails of one of the assistants in the hospital who is in the habit of making daily use of sublimate solutions. The first set were inoculated with scrapings without any preliminary treatment of the hands; the second after thorough scrubbing with soap and warm water by means of a sterilized brush; and the third after washing the hands, subsequent to the scrubbing with soap and water, with a solution of sulphide of ammonium, which brought out a deep brown or black color of the epidermis and nails. You will observe that all of the tubes of the first set contain a large number of colonies, that two of the tubes of the second set contain no colonies and two contain from three to ten colonies, and that the tubes of the third set contain from twenty to one hundred colonies.

In this second series of agar roll cultures, intended to show the results of Fürbringer's method, the experiment has been made with hands which have not previously been in sublimate solution. The first set of tubes were inoculated with scrapings from beneath the nails without preliminary treatment; the second after thorough scrubbing with soap and water; the third after the use of alcohol, and then washing in sublimate 1-500 for two minutes; and the fourth after washing the hands in sulphide of ammonium, which turned them dark in color. Each step was carried out strictly according to Fürbringer's directions, and double the amount of time which he recommends given to each step. The sublimate was freshly prepared in distilled water. The hands were well-washed in sterilized water and then dried with a sterilized cloth before inoculating the third set of tubes. Each set was inoculated with the scrapings from beneath and around the nails and placed in the thermostat at 37° C. You will observe that the first set of tubes are crowded with colonies; that the second contain a smaller number, but still a good many colonies, from twenty to over a hundred; that the third set are sterile, save one colony in one of the tubes; and that the fourth set contain some colonies in each tube, varying from three to twenty-five. Fürbringer inferred from such results as those in the third set of tubes that the sublimate had actually killed all of the bacteria in the superficial layers of the epidermis and beneath the nails, whereas, if he had precipitated the mercury with ammonium sulphide as was done before inoculating the fourth set, he would have learned that this is not the case. It is fair to say that we have sometimes obtained sterile tubes by Fürbringer's method, even after precipitation with ammonium sulphide, and we do not intend to condemn the method as not a good one, but only as not the best, and not accomplishing all that is claimed for it. As has already been said, it may be that it is sufficient to inhibit the growth of the bacteria in the way that is done by the sublimate, but it is not justifiable to infer that because the bacteria will not grow in our culture media they may not grow in the animal body. It is also to be considered that the sublimate is capable of attenuating or destroying the pathogenic power of some bacteria. But even after giving due weight to these considerations, that method which actually kills the bacteria is to be preferred if it is applicable.

The third series of tubes has been inoculated with scrapings from beneath the nails after disinfecting the hands with lysol; and the fourth after disinfection with peroxide of hydrogen, the preliminary scrubbing with soap and water being as in the other experiments. You will observe that neither of these agents has accomplished complete disinfection, there being a few colonies in most of the tubes, the most in the tubes after using peroxide of hydrogen. The hands were disinfected with lysol in the manner recommended by Gerlach.

On the other hand, in this fifth series of tubes inoculated with scrapings from beneath the nails after disinfection with permanganate of potash and oxalic acid in the manner described in my paper before the Congress in Washington, you will observe that all of the tubes are sterile with the exception of one colony in one of the tubes. We, therefore, have adopted in the hospital this method of disinfection of the skin. Whether there may lurk some fallacy in the conclusions which we have drawn from our experiments with this method analogous to that in previous experiments with corrosive sublimate I cannot say, but if so, we have not been able to detect it.

Our experiments with methods of cutaneous disinfection, as well as our observations on the bacteria in wounds treated antiseptically, have led us to a study of the bacteria in the skin. The microorganisms in the human epidermis have been studied by a number of investigators, as Bizzozero, Bordoni-Uffreduzzi, Unna, Maggiora, Mitmann and Preindlsberger, and a large number, over a hundred different species, have been more or less perfectly described. Our purpose has been not to make a complete study of the bacterial flora of the skin, although we do not regard such a study in the light of Fürbringer's characterization of it as a "zeitraubende, geistestödtende, wenig fördernde Beschäftigung," but rather to learn something about the frequency of occurrence of the most common pyogenic cocci, about the most common bacterial inhabitants of the epidermis and about the possibility of the presence of bacteria in the layers of the epidermis not reached by existing methods of cutaneous disinfection.

In conformity with Fürbringer's statements as to the relation between the occupation of an individual and the bacteria present beneath the nails, we have found *Staphylococcus pyogenes aureus* only very exceptionally in the epidermis or in the subungual dirt of those who have had nothing to do with suppurating wounds, abscesses or other surgical cases, but on the other hand have found this organism repeatedly on the skin or beneath the nails of surgeons or their assistants. The bearing of these observations are sufficiently apparent to need no further emphasis.

We have found a white staphylococcus far more frequently than any other organism in the epidermis and beneath the nails after the superficial bacteria have been removed by scrubbing the hands with soap and water or by incomplete disinfection, as by washing with sublimate. Unna and others who have studied by culture methods and have paid attention to the relative frequency of the different kinds of bacteria on the skin have also noted the frequency of occurrence of a white coccus liquefying gelatine. Unna regards this most common coccus as *Staphylococcus pyogenes albus*. We have observed that this common epidermal staphylococcus liquefies gelatine and coagulates milk more slowly than the typical *Staphylococcus pyogenes albus*, and that a centimeter and more of bouillon cultures can be injected without effect into the veins of rabbits. Whether this occurs as a distinct species or only as a modified form of *Staphylococcus pyogenes albus,* we have not been able definitely to decide, but I have proposed for it, provisionally, the name *Staphylococcus epidermidis albus.* Other white cocci also occur in the skin. We have met, several times, forms corresponding to *Micrococcus cereus albus* (Passet), *Micrococcus candicans* (Flügge) and *Micrococcus albus liquefaciens* (v. Besser). Von Besser has found in the normal air passages a white staphylococcus resembling *Staphylococcus pyogenes albus* save by the absence of pathogenic properties. In general, the classification of the white liquefying micrococci is difficult and unsatisfactory. There are differences in the rapidity of liquefaction of gelatine by our *Staphylococcus epidermidis albus* and occasionally we have isolated forms which correspond in mode of growth to *Staphylococcus pyogenes albus,* but have not isolated any with the pathogenic powers of this coccus, so far as we have been able to test this point. We have also occasionally found in the epidermis a staphylococcus resembling closely, in the appearance of its colonies on agar and its scarcely visible growth on potato, the typhoid bacillus.

We regard as the most important outcome of our studies of the bacteria of the skin the demonstration of the presence of *Staphylococcus epidermidis albus,* less frequently of other bacteria, in layers of the skin deeper than can be reached by existing methods of disinfection of the skin. The significance of this fact is apparent when I tell you that this same coccus is the one which we have found in a very large number of laparotomy wounds treated by the most careful antiseptic or aseptic methods and that it is the most frequent cause of stitch abscesses. We have found it so often in the deeper layers of the epidermis that we are inclined to consider it a regular inhabitant of the skin. It has only feeble pathogenic power ordinarily, but it is capable of causing suppuration. The injurious influence of a drainage tube in checking the repair of fresh healthy wounds is illustrated by the fact that it is chiefly in wounds containing drainage tubes that we have observed suppuration to result from the presence of this organism, and that often the suppuration is limited to the track occupied by the tube. This coccus is found not only in stitches around which suppuration has occurred, but also in those without any visible inflammatory reaction around them.

I show you here a number of cultures of this organism. These three cultures were made in the following way: the skin of a patient before an operation was thoroughly disinfected by the method already described, so that scrapings from the surface afforded no growth, then by means of a sterilized instrument; sterilized silk threads (tested by cultures), were drawn from one to five or six times through the disinfected skin and these silk threads were then dropped into liquefied agar, the tubes were agitated and then rolled on ice. You will observe that several white colonies (varying from two to ten), and coalescent colonies have developed, especially along the sides of the thread. The remaining five agar roll cultures have been inoculated with silk sutures removed from the skin, four to seven days after antiseptic laparotomies, pursuing in each case a perfectly aseptic course and without any evidence of inflammatory reaction around the stitches. Each of these tubes contains colonies of the white staphylococcus, varying from six to fifty in number.

We have found also other bacteria, and twice *Staphylococcus pyogenes aureus,* in skin stitches withdrawn after antiseptic operations, but *Staphylococcus epidermidis albus* is by far the most common. To judge from the number of colonies developed from sterilized threads drawn through the skin after complete disinfection of the surface it would seem as if the white staphylococcus were not particularly abundant in the epidermis. Further investigations, which must be microscopical, are necessary to determine the exact part of the epidermis or hair follicles or glandular appendages of the skin occupied by these bacteria, and here the methods recently described by Unna are likely to be of service.

I hardly need to say that these observations on the bacteria of the normal skin, and the depth to which they penetrate, indicate that the skin of the patient may be a source of wound infection and that the surgeon should take greater precautions than has hitherto been customary, to guard against this danger. Dr. Halsted, in view of our results on cutaneous disinfection, has discarded for the most part the use of cutaneous sutures, and is very well satisfied with the results obtained by bringing the edges of wounds together by subcutaneous sutures.

I have endeavored, gentlemen, to bring before you, although in a disconnected way, a few of the improvements in antiseptic surgery since Lister's pioneer work, as they present themselves to one looking at the subject from a bacteriological point of view. Surely progress has been great, but there is no reason to suppose that the ideal has yet been reached and that there is not room for further advance. Even in directions where it now seems to us that the goal is attained we must be guarded in our predictions, lest we make ourselves as ridiculous to future generations as to us seems the leading surgeon of his day, Boyer, when in the early part of this century he wrote " Surgery in our day has made the greatest progress and appears to have attained, or nearly so, the highest perfection of which it is capable."

GENERAL BACTERIOLOGY OF SURGICAL INFECTIONS[1]

Members of each of the three groups of pathogenic microorganisms—bacteria, fungi, and protozoa—may cause surgical infections. Fungi and protozoa, however, are far less commonly concerned in these infections than are bacteria. In a general consideration of the conditions of surgical infection bacteria are the organisms which require chief attention, so that the subjects to be considered in this article can be appropriately included under the designation "General Bacteriology of Surgical Infections."

The term "general bacteriology" is here used in distinction from "special bacteriology" to designate the general relations of bacteria to surgical infections. It is not deemed necessary in this article to enter into a detailed consideration of the morphological and cultural characters of bacteria. This subject is fully treated in works especially devoted to bacteriology.

Infectious diseases which require especial consideration by the surgeon include, on the one hand, many specific infections, such as tuberculosis, tetanus, glanders, anthrax, and actinomycosis, and, on the other hand, traumatic and other inflammatory and septic infections caused by various widely distributed bacterial species.

The specific infectious diseases, such as tuberculosis, tetanus, etc., are for the most part sharply differentiated by their anatomical and clinical characters, and are caused by microorganisms which are constantly and exclusively associated with their respective diseases.

On the other hand, the common traumatic infections and other surgical inflammations and septic processes do not present equally sharp and definite differential characters, and apparently identical or similar pathological processes belonging to this group of affections may be caused by various microorganisms. Thus we do not find in such diseases as septicaemia, pyaemia, abscesses, osteomyelitis, puerperal fever, or other septic and localized inflammations, or, in general, in the infections of wounds, any single bacterial species constantly and exclusively associated with each of these affections, but each disease of this group may be caused by more than one species of microorganism.

The *etiology* of these common septic and inflammatory affections presents for our consideration many problems quite distinct from those pertaining to the causation of the specific infections. The views now held as regards sources of infection, operative procedures, and the management of wounds have been developed largely as the result of investigations concerning the relation of bacteria to traumatic infections.

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[1] In: Syst. Surg. (Dennis), Phila., 1895, I, 249-334.

It is important that the surgeon should become familiar with the various kinds of bacteria concerned in surgical infections, with their distribution on exposed surfaces of the body and in the outer world, with the ways by which they may enter and be discharged from the body, with the various conditions which favor their invasion and multiplication in the body, with their pathogenic manifestations, and with the means of combating them. It is proposed in this article to present the more important considerations pertaining to these subjects so far as they do not fall more appropriately for their treatment to other sections of this work.

We shall consider first the distribution of bacteria on exposed surfaces of the body, having especially in view its surgical bearings. Knowledge of the distribution of pathogenic bacteria is of importance in the study of the causation of surgical infections, as indeed of all infectious diseases.

Bacteria of the Skin

There are various questions of surgical interest relating to the bacteria of the skin. The destruction of the surface bacteria both on the hands of the operator and his assistants and over the field of operation in the patient is of fundamental importance in surgical technique. It is important to determine the possibilities of danger from infection by bacteria commonly or occasionally found in or on the skin. Ignorance of the bacterial flora of the normal skin has led some investigators to erroneous interpretations of their observations as to the source of bacteria found in wounds treated aseptically or antiseptically, and as to the presence of supposed specific pathogenic bacteria in certain cutaneous diseases.

The microorganisms of the human skin have been studied by several investigators, of whom may be mentioned Bizzozero, Bordoni-Uffreduzzi, Unna, Maggiora, Mittmann, Fürbringer, Preindlsberger, Robb and Ghriskey, and Welch.[2]

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[2] Bizzozero, Virchow's Archiv, Bd. 98; Bordoni-Uffreduzzi, Fortschritte der Medicin, 1886, p. 151; Unna, Monatshefte für praktische Dermatologie, 1889, 1890, 1891; Maggiora, Giornale della R. Società d' Igiene, 1889; Mittmann, Virchow's Archiv, Bd. 113; Fürbringer, Desinfektion d. Hände d. Arztes, Wiesbaden, 1888; Preindlsberger, Zur Kenntniss der Bacterien des Unternagelraumes u. s. w., Wien, 1891; Robb and Ghriskey, Johns Hopkins Hospital Bulletin, April, 1892; Welch, Trans. of the Congr. of American Physicians and Surgeons, vol. ii., and Maryland Medical Journal, Nov. 14, 1891.

As the skin is exposed to contamination from the air and all sorts of sources, it is evident that there is scarcely any limit to the number of species of bacteria which may possibly be found on the skin. Most investigators of this subject have not had the patience or have not thought it worth while to attempt to identify or to describe all of the various kinds of bacteria developing in cultures from the surface of the skin. Mittmann mentions seventy-eight different species of cutaneous bacteria, of which fifty-six were cocci. His descriptions, however, are so imperfect as scarcely to serve for the identification of the species. Preindlsberger describes thirty-two species, of which twenty-eight were cocci. Maggiora isolated twenty-nine microorganisms, of which twenty-two were bacteria, three budding fungi, and four moulds. Most of these bacteria are such as are often found in the air or on external objects. Cocci are usually found much more abundantly than bacilli in cultures from the skin.

Great variations exist in different cases as to the kinds and the number of bacteria found on the skin. Sometimes one species predominates over the rest, indicating that it has multiplied and overgrown other bacteria. The conditions in general are not favorable for the growth of bacteria on the surface of the skin, but under the nails and in situations where moisture collects, as in the axillae, the groins, and between the toes, there may be abundant multiplication of certain species of microorganisms. Although bacteria predominate, budding and mould fungi are often present. The large number of microorganisms which accumulate beneath the nails is a matter of surgical importance. From a minute particle of material from this situation sometimes as many as two thousand to five thousand colonies develop in culture media, although usually the number is much less, and may be very small indeed, perhaps not more than three or four colonies.

The writer in 1891 was the first to call attention to the fact that, although in general the bacterial flora of the skin is inconstant and indefinite in its special characters, there is one bacterial species, to which he gave the name of *Staphylococcus epidermidis albus,* which is found with such regularity in cultures from the skin that it may properly be regarded as a regular inhabitant of the normal skin, just as *Bacillus coli communis* is a regular inhabitant of the intestinal canal. The principal data relating to this staphylococcus were established by the researches of Robb and Ghriskey. We consider this coccus to be a variety of *Staphylococcus pyogenes albus.* It is possessed of feeble pyogenic power, and usually liquefies gelatin and coagulates milk more slowly than the ordinary white pyogenic staphylococcus. For these reasons, but especially to emphasize the epidermis as a normal habitat for this organism, we applied the designation mentioned.

One of the chief points of interest relating to this coccus is that it is very often, probably regularly, present in layers of epidermis along the hair-shafts, deeper than can be reached by any known means of cutaneous disinfection save the application of heat. After complete sterilization of the surface of the skin, so that scrapings are sterile when inoculated into culture media, the presence of this white coccus can still be demonstrated by making cultures from sutures passed through the skin or from excised pieces of skin.

Staphylococcus epidermidis albus is usually innocuous. It is found frequently in aseptic wounds of the skin without causing suppuration or any trouble. The source of this coccus in aseptic wounds does not seem to be known to many who have made bacteriological examinations of such wounds; thus Büdinger,[3] who examined in 1892 twenty operative wounds which healed by first intention in Billroth's clinic, could not suggest any other origin for its presence than the air, and C. Fraenkel[4] suggests that it is brought to the wound by the blood current—suggestions which were rendered quite unnecessary by our previous researches.

Although this white epidermal staphylococcus is often found in wounds without any disturbance in the process of healing, it may be the cause of some disturbance, characterized especially by elevation of temperature and moderate suppuration. This is particularly likely to be the case when there is necrotic or strangulated tissue in the wound or when foreign bodies have been introduced into the wound. It is a common, although not the sole, cause of stitch abscesses, and it is prone to travel down along the sides of a drainage tube, and under these circumstances may cause the wound to suppurate. It is often associated with other pyogenic cocci in cutaneous inflammations. We can now understand how, without any flaw in the antiseptic technique of the surgeon, this microorganism may be present in wounds, and we have a satisfactory explanation of the frequent occurrence of stitch abscesses.

This white skin coccus is often present in cultures from blood obtained by puncture of the human skin and in cultures from the sweat after complete disinfection of the surface of the skin. Some observers seem to have supposed that when *Staphylococcus albus* is demonstrated under these conditions its presence in the circulating blood or its excretion by the sweat-glands can be inferred. But it is evident that such an inference is unwarranted without additional proof.

There are various other white cocci, and also several species of yellow cocci, both liquefying and nonliquefying, which are frequently found in cultures from the skin. Some of the yellow cocci can readily be mistaken

[3] Büdinger, Wiener klin. Wochenschr., 1892, Nos. 22, 24, 25.

[4] C. Fraenkel, Baumgarten's Jahresbericht. 1892. p. 28.

for *Staphylococcus pyogenes aureus,* unless they are carefully studied in culture media. *Staphylococcus pyogenes aureus* may be found on the skin, as will be mentioned presently.

The kinds and the number of bacteria found upon exposed parts of the skin vary considerably according to the habits and the occupation of the individual. Of especial interest in this connection are the results of the examination of the skin of surgeons and others who come into contact with infected persons either during life or at the autopsy table. We have found only exceptionally *Staphylococcus pyogenes aureus* upon the hands of those who do not come into proximity to surgical or infected cases, whereas we have many times found this microorganism upon the hands of surgeons, their assistants, and surgical nurses. In examining the hands of those who use corrosive sublimate as a disinfectant it is necessary first to neutralize the sublimate with ammonium sulphide, as we have found that the sublimate may prevent the development of cutaneous microorganisms with which it has come into contact, although it has not killed them; and this restraining influence may be manifest days, and even weeks, after the application of the sublimate.

The length of time that the yellow pyogenic staphylococcus may persist upon the hands varies, and doubtless largely, according to the methods and extent of cleansing the skin. It certainly may persist for several days, although it may disappear in a few hours. It does not seem usually to grow down, as does the white epidermal coccus, into the deeper layers of the skin, so that ordinary methods of disinfection of the skin are likely to remove or destroy this organism. By rubbing or by the application of pressure *Staphylococcus aureus* may, however, be pressed into the deeper layers, particularly into the hair-follicles, and there cause furuncles, as has been shown by the experiments of Garré, Schimmelbusch, Wasmuth, and others. The view which has been advocated by some writers that it requires more thorough disinfection to remove or destroy pathogenic bacteria accidentally or intentionally applied to the skin than to kill the ordinary bacteria of the skin is not supported by experiments. The healthy skin in general is not a favorable resting place or breeding place to secure the long persistence of pathogenic bacteria, with the exception of the white epidermal coccus, which possesses relatively little pathogenic power under ordinary circumstances.

Streptococcus pyogenes has been found less frequently than *Staphylococcus aureus* in cultures from the skin, and here too chiefly in cultures from the skin of infected patients or of those who have been in proximity to them. It is well to bear in mind that these pyogenic cocci are not necessarily limited to the immediate neighborhood of an infected wound or focus, but may occur on other parts of the body, as well as in the air, or on objects which have been near the patient. Thus Preindlsberger found the aureus in the dirt beneath the finger-nails of a patient with fracture of the femur, and *Streptococcus pyogenes* in the same situation in a patient with osteomyelitis femoris for which necrotomy had been performed. The complete disinfection of an infected wound, even if that were possible, would not therefore furnish a guarantee that pyogenic cocci were not present upon the surface of a patient's body in other situations.

Bacillus pyocyaneus is a common parasite upon the human skin. Mühsam[5] found it in the axilla and in the anal and inguinal folds of healthy persons in 50 per cent of the cases examined. Probably local conditions were concerned in these observations, as others have not found this organism upon the healthy skin with such frequency. Although this organism may manifest important pathogenic activities, its presence in wounds is usually made evident chiefly by the green or blue discoloration of the dressings. It was formerly supposed to enter the wound from the air, but it doubtless is often derived also from the skin of the patient.

The common intestinal bacterium, *Bacillus coli communis,* is of course often present on the skin about the anus. In abscesses in this situation it is often found either alone or associated with other bacteria. The colon bacillus may also be found upon the skin in other parts of the body. It is a widely distributed bacterium outside of the animal body. It has repeatedly been found in wounds in different parts of the body.

Skin contaminated with the soil, which, as is well known, contains in many situations abundant bacilli of tetanus and of malignant oedema, is likely to present these microorganisms. This contamination relates, of course, especially to the hands, and in the case of those who go barefoot or have holes in their shoes also to their feet. Maggiora was able to demonstrate the bacillus of malignant oedema in scrapings from between the toes of a person who had walked for half an hour in a garden with a torn shoe. It is not therefore in all cases necessary to suppose that the tetanus bacillus enters a wound from the object which causes the wound, for this bacillus may previously have been attached to the skin. It may in this connection be mentioned that the faeces of herbivorous animals often contain the tetanus bacillus, and in a condition more likely to produce tetanus than when the bacillus is obtained from the soil. Buday[6] has reported a case of tetanus fatal in twenty-four hours which followed the smearing of a wound with faeces.

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[5] Mühsam, cited by Schimmelbusch, Samml. klinischer Vorträge von Volkmann, Serie 3, Heft. II, No. 62.

[6] Buday, Pester Med.-chir. Presse, 1894, No. 19.

Bacillus smegma may be considered in connection with the cutaneous bacteria. This bacterium is usually present in the smegma, and may be found about the penis, scrotum, vulva, and anus. Attention was first called to this bacillus by Alvarez and Tavel and by Matterstock in 1885, on account of its resemblance in morphology and staining reactions to Lustgarten's bacillus, which at that time was thought by its discoverer to be the specific cause of syphilis. Greater practical importance, however, belongs to the smegma bacillus at present on account of the possibility of mistaking it from its staining properties for the tubercle bacillus, and there is reason to believe that such mistakes have been made in examinations of the urine and of secretions or exudates about the external genitals and the anus. The smegma bacillus resembles the tubercle bacillus in the property of retaining the staining dye after such application of acids and alcohol that all known bacteria except the tubercle bacillus, the smegma bacillus, and the leprosy bacillus are decolorized. This property probably does not inhere in the smegma bacilli as such, but is due to the presence of chemical constituents of the smegma, although this point is not positively settled. In the opinion of the writer this peculiar staining reaction does not belong to only a single species of bacillus in the smegma, but to several, so that it is more proper to speak of smegma bacilli with this reaction. Mistakes are particularly likely to occur when the handy and popular Gabbett's stain for the tubercle bacillus is employed. The usual statement is that the smegma bacilli can be distinguished from the tubercle bacillus by less resistance to decolorizing agents, particularly to nitric acid, hydrochloric acid, and alcohol, also to counterstains; and this often holds true. Nevertheless, smegma bacilli are sometimes encountered which are as resistant to these decolorizers as are tubercle bacilli. Especial attention should be given to the morphological appearances, as the size and shape of the bacilli often suffice for the distinction, although there is considerable diversity as regards this feature between the different smegma bacilli which resist decolorization. Although smegma bacilli may be present with pathogenic bacteria in lesions around the genitals and anus, they are not known to possess pathogenic capacity.

Many bacteria are attached to the hairs of the body, and particles containing bacteria may readily be detached from the hair. Robb has studied the bacteria which fall off from the hair of the head by movement or by combing the hair. They are identical with those found on the skin, as might be expected. Haegler has cultivated pyogenic staphylococci from the hair of surgeons, and calls attention to the possibility of such cocci falling from the hair into a wound or upon objects coming into contact with the wound during an operation. Wright has found the diphtheria bacillus on the hair of nurses in attendance on cases of diphtheria.

The cerumen is rich in bacteria. Rohrer[7] isolated sixteen species of microorganisms from the cerumen of fifty cases, but he has not attempted to identify any of these with previously known bacteria, and his statements as to the existence of pathogenic bacteria in the cerumen are not based upon conclusive observations.

Bacteria of Exposed Mucous Surfaces

The way is open for the access of microorganisms to mucous membranes which cover parts which communicate with the outer world through the external orifices of the body. So far as temperature, moisture, and the presence of nutritive pabulum are concerned, the conditions are manifestly more favorable for the growth of bacteria upon mucous surfaces than upon the dry skin. These relatively favorable conditions for the development of microorganisms upon mucous membranes are, however, counteracted in large measure by various mechanical and chemical influences which prevent the prolonged survival of most of the bacteria which may enter through the external orifices of the body. There are, however, many bacteria which may multiply, or persist for a long time or indefinitely, upon certain mucous membranes in health, particularly those of the alimentary canal and of the upper respiratory tract, and there are some bacterial species which find their natural home here. Some pathogenic bacteria may live upon certain mucous membranes without doing harm.

The study of the bacterial flora of exposed mucous membranes in health and in disease has brought to light many points of surgical interest.

Conjunctiva.—The bacteriology of the conjunctiva has been investigated by many ophthalmologists, of whom may be especially mentioned Fick, Weeks, Leber, Felser, van Genderen Stort, Gombert, Bernheim, Hildebrandt, Franke, Marthen, and Bach.[8]

When one considers the exposed situation of the conjunctiva, it is surprising to find how small is the number of bacteria usually present in the conjunctival sac. Fick found upon microscopical examination of fifty healthy conjunctivae, bacteria in only eighteen, although in another series in which forty-nine healthy eyes of paupers were examined bacteria were missed in only six. A negative microscopical examination, however, indicates only that the number of bacteria is small, as then their presence may readily be overlooked without the aid of cultures. As a matter of fact, cultures from the healthy conjunctival sac usually furnish colonies of bacteria. Their number may be considerable, but it often happens that not more than three of four colonies develop from a loopful of fluid from the conjunctiva, and it is not very uncommon for culture media inoculated in this way to remain sterile. It is to be assumed that the conjunctival sac ordinarily contains bacteria. The secretion within the lachrymal glands is sterile.

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[7] Rohrer, Archiv f. Ohrenheilk., Bd. XXIX.

[8] L. Bach, "Ueb. d. Keimgehalt des Bindehautsackes," Archiv f. Ophthalmologie, Bd. XL, p. 130. This article contains references to the other articles cited in the text.

Bach describes twenty-six species of bacteria isolated in pure culture from the healthy or diseased conjunctiva. Of these, ten are liquefying cocci, nine nonliquefying cocci, five liquefying bacilli, one nonliquefying bacillus, and one cladothrix. Ten of the twenty-six bacteria were found to be more or less pathogenic when inoculated into the rabbit's cornea. Pink yeast and mould fungi have also been cultivated from the conjunctiva.

Bach considers that of these various bacteria only *Staphylococcus pyogenes aureus* and *albus* and *Streptococcus pyogenes* are demonstrated to be pathogenic for man, although the possibility that others in the list may be pathogenic for man must be admitted. In a few instances *Staphylococcus pyogenes aureus,* and in more the albus, have been cultivated from the healthy conjunctiva. Cultures of *Staphylococcus pyogenes aureus* have been introduced into the healthy conjunctival sac of man and animals without causing inflammation.

Inasmuch as many microorganisms must enter the conjunctival sac from the air, the edges of the eyelids, and from contact with the fingers and other objects, and as relatively few bacteria are found ordinarily in cultures from this part, it is evident that there must be some very efficient mechanism which rids the conjunctiva of most of the bacteria which enter. There are two principal agencies by which this may be accomplished—namely, mechanical removal through the nasolachrymal duct, and the germicidal action of the lachrymal and conjunctival secretions. Some investigators have attached the greater importance to the former, others to the latter, of these agencies. Experiments have shown that each may be operative, but the mechanical removal has been shown to be especially efficient and prompt in its action.

Van Genderen Stort has made experiments upon rabbits, and Bach upon man, by dropping pure liquid cultures of easily identified bacteria, such as the Kielwater bacillus, *Bacillus coli communis, Staphylococcus aureus,* into the conjunctival sac, and then determining by cultures the length of time during which they can be demonstrated. Van Genderen Stort found after fifteen minutes the inoculated bacteria reduced to a small number, and at the end of an hour they had nearly or entirely disappeared from the conjunctival sac. Cultures from the nose showed that they had been carried down the nasolachrymal duct, and that after five minutes they were abundant in the cultures from the nasal cavity. Identical results were obtained by Bach in his experiments on human beings. In the experiment of Bach with *Staphylococcus aureus,* however, which was introduced in such number that the immediate plates from the conjunctiva contained countless colonies, there were still seventy-four colonies in the plates made after twenty-four hours.

The same experiments were made after ligation of the nasolachrymal duct. Under these circumstances the inoculated bacteria also disappeared from the conjunctival sac, but more slowly than when the duct was open. Still, after an hour most of the bacteria had been removed. It was found that they had been carried away by the lachrymal secretion which flowed over the eyelids. If the eyelids were closed by a bandage, the bacteria, with open nasal duct, rapidly pass down into the nose, and they did not appear to pass out between the closed eyelids. When, however, the duct was closed, they appeared abundantly in the bandage.

Bernheim has demonstrated that the tears possess considerable bactericidal power over certain species of bacteria, and his results have been in part confirmed by the experiments of Marthen, Bach and others. *Staphylococcus pyogenes aureus* is killed in moderate number, and the typhoid bacillus in larger number, by the lachrymal secretion. We must therefore assign importance to this chemical action, as well as to the mechanical removal, in ridding the conjunctiva of invading bacteria. Neither of these agencies suffices to remove all of the bacteria. Some bacteria grow readily in the fluid of the conjunctiva. As has already been mentioned, the conjunctiva nearly always contains some bacteria. The orifices of the Meibomian glands, the cilia, and the edges of the eyelids usually contain many bacteria, and these of course may readily enter the conjunctival sac.

Bach was unable to demonstrate that bacteria introduced into the nasal cavity ever made their way up the nasal duct to the eye.

It is difficult to obtain complete disinfection of the conjunctival sac. The number of bacteria may be greatly reduced, either by the application of antiseptics or by simple mechanical cleansing, combined with irrigation by an indifferent fluid, such as sterilized salt solution. Bach obtained the best results by the latter procedure, by which in sixteen out of forty-two cases he rendered the conjunctiva sterile.

The importance of familiarity with the saprophytes to be found on exposed mucous membranes is illustrated by the history of the so-called xerosis bacillus. This bacillus was discovered by Neisser in 1882 in xerosis of the conjunctiva, and was regarded by him as the cause of this disease. This conclusion was adopted by several subsequent investigators, who found this bacillus constantly present in xerophthalmia. This same bacillus has, however, been demonstrated by Schreiber, working under Neisser's direction, in various other affections of the eye, and also, although in small number, in the normal conjunctival sac. Neisser has therefore given his assent to the conclusion expressed by Schreiber[9]: "The so-called xerosis bacilli are to be regarded as saprophytes which are often present in the conjunctiva and its secretion, both in diseased and healthy eyes, and they play no special rôle either in xerosis or in other diseases of the eye."

The xerosis bacillus belongs to an interesting group of bacteria which are characterized by remarkable irregularities in size and shape—so-called involution forms—and by irregularities in staining, especially by the presence of deeply staining isolated granules. It is not positively proven to form spores, although some of the isolated granules are interpreted as such by Ernst and by Neisser. To this same group belong the diphtheria bacillus of Löffler and the so-called pseudodiphtheria bacilli.

Mouth and Pharynx.[10]—All of the microorganisms which may be present in the air, food, and ingested fluids may appear temporarily in the mouth. The number of bacteria which have been cultivated from the human mouth is very large. Miller has isolated over one hundred species. Freund has cultivated eighteen different chromogenic microorganisms from the mouth.

It is important to distinguish between the countless bacteria which may appear as transient visitors in the mouth and those which find their permanent home there. A remarkable peculiarity of the constant inhabitants of the saliva is that most of them will not grow in our artificial culture media. This produces often a striking discrepancy between the results of microscopical examination of the buccal secretions and those obtained by cultures. Cover-slip specimens may show an enormous number of bacteria, when cultures made from the same material may show very few or even no colonies.

Miller enumerates the following as the constant buccal bacteria: *Leptothrix buccalis innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum, Spirochaete dentium.* None of these have been artificially cultivated. *Bacillus buccalis maximus* and *Iodococcus vaginatus* are stained violet by iodine solution. These bacteria are often present, with others, in carious teeth, in abscesses communicating with the mouth and pharynx, and in exudates on the mucous membranes of these parts, but they have not been proven to be pathogenic.

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[9] Schreiber, Fortschritte der Medicin, 1888, p. 656.

[10] Miller, Die Mikro-organismen der Mundhöhle, Leipzig, 1889; David, Les Microbes de Bouche, Paris, 1890. These valuable works present the most important results hitherto obtained by the investigators of the microorganisms of the mouth.

The frequent presence of pathogenic bacteria in the healthy mouth is of great practical importance. The following pathogenic bacteria have been found repeatedly in this situation: *Micrococcus lanceolatus, Streptococcus pyogenes, Staphylococcus pyogenes aureus* and *albus, Micrococcus tetragenus, Bacillus pneumoniae* of Friedländer, *Bacillus crassus sputigenes* of Kreibohm, *Bacillus coli communis.* Biondi, Miller, Kreibohm, Galippe, and others have found in the mouth additional pathogenic bacteria in isolated cases, mostly, however, with some morbid condition.

Micrococcus lanceolatus was discovered by Sternberg in his saliva in 1880. Many names have been given to this bacterium, the more common synonyms being *Diplococcus pneumoniae,* pneumococcus of Fraenkel and Weichselbaum, *Diplococcus lanceolatus,* micrococcus of sputum septicaemia, and *Micrococcus pneumoniae cruposae* (Sternberg). This microorganism was found by Netter in a virulent condition in 15 to 20 per cent of the healthy persons whom he examined. It varies markedly in virulence, and it is probably present in a nonvirulent condition in many cases. Indeed, Kruse and Pansini believe that *Micrococcus lanceolatus* is a regular inhabitant of the human mouth, although it is present in a virulent state in only about one out of five or six persons. As the lanceolate coccus, especially when its virulence is weak or absent, may grow in chains and present cultural characters of *Streptococcus pyogenes,* it is often difficult, if not impossible, to distinguish between these bacteria.

The chief interest attaching to the frequent presence of *Micrococcus lanceolatus* in the healthy mouth is that this bacterium is the cause of lobar pneumonia and of many cases of bronchopneumonia. It may also be concerned in local inflammations of the throat, and as a primary or secondary invader may cause serositis and localized inflammations in various parts of the body; but *Streptococcus pyogenes* is a more common cause of these lesions.

Various virulent and nonvirulent streptococci have been found in the mouth, both short chained streptococci and long chained forms, corresponding to the two varieties, *Streptococcus brevis* and *Streptococcus longus.* The distinctions upon which these varieties were established by Von Lingelsheim are often inconstant, and do not serve for a sharp differentiation. The chief interest belongs to the presence in the mouth and pharynx of *Streptococcus pyogenes.* Netter found *Streptococcus pyogenes* in seven out of one hundred and twenty-seven healthy mouths examined; that is, in 5.5 per cent of the cases. Dörnberger found streptococci in the mouths of healthy children in 45 per cent of the ninety-four cases examined. Widal and Besançon found streptococci constantly and in large number in the mouths of twenty healthy persons, and still more abundantly in the mouth and pharynx of forty-nine persons affected with various diseases.

It often requires a painstaking examination to detect this streptococcus. The colonies are minute and pale gray, and in a plate crowded with other more striking colonies the former may escape recognition unless especial attention is given to them. If the secretions of the healthy mouth and throat be carefully examined both by cover-slip preparations and by agar plate cultures, streptococci will be found with great frequency, if not regularly, although, as already mentioned, it is by no means easy to distinguish some of the streptococci from chain forms of *Micrococcus lanceolatus*.

The number of streptococci is increased and their detection is much easier in most inflammatory conditions of the tonsils and pharynx. They are commonly associated with the diphtheria bacillus in diphtheria, and they are capable of causing all grades of tonsillitis and pharyngitis, from slight erythematous forms to pseudomembranous and necrotic inflammations.

Streptococci cultivated from the healthy mouth usually have little or no virulence as tested upon animals, and the same is often true of streptococci cultivated from the throat in various infections, local and general, although in these cases they are more likely to be pathogenic for animals.

Streptococcus pyogenes is a common and dangerous invader of the deeper air passages and lungs and of the internal parts of the body. The portal of entry is often the tonsils and throat, and predisposing causes are inflammations and other lesions of these parts, particularly when combined with other infectious diseases and constitutional disturbances. Under conditions little understood the mouth streptococci may acquire enhanced virulence.

The influence of predisposing causes as a factor in the etiology of infections is well exemplified by the fact that healthy mucous membranes harbor very frequently such pathogenic germs as *Micrococcus lanceolatus* and *Streptococcus pyogenes*. These bacteria often, moreover, cause no serious disturbance in the repair of wounds and injuries involving the mouth and nasopharynx, although they must gain access to such wounds. They are, however, a standing menace in surgical operations involving these parts, and they may seriously interfere with the healing of such wounds, or may under these circumstances set up pneumonia and general infection.

Staphylococci are found often in the healthy mouth and the throat, but the genuine pyogenic staphylococci do not appear to be present with great frequency. Vignal, Netter, and Miller met *Staphylococcus pyogenes aureus* only in a comparatively small number of cases in their bacteriological examinations of the healthy mouth. It is found more frequently in various inflammations of the mucous membranes of this region, but it plays no such important rôle in these as does *Streptococcus pyogenes*.

White liquefying cocci, often described as *Staphylococcus pyogenes albus*, are found oftener than *Staphylococcus aureus* in the mouth and throat. Some of these have been shown to possess pyogenic power, and may be accepted as *Staphylococcus pyogenes albus*, but others are devoid of such power.

According to the statements of Biondi, Miller, and others, *Micrococcus tetragenus*, which was discovered by Koch and Gaffky in a phthisical cavity, is often present in the mouth. In a considerable number of cases examined by the writer it was absent. The frequency with which certain bacteria are present in the mouth probably varies considerably in different regions and according to the class of cases selected for examination. There are various species of tetragenous cocci. The pathogenic form of Koch and Gaffky is designated by Boutron as *Micrococcus tetragenus septicus*. This organism is present more frequently in abscesses in the neighborhood of the mouth and throat, particularly those connected with carious teeth, than in abscesses in other parts of the body, although even in the former it is rarely present.

The virulent diphtheria bacillus was found by Park and Beebe[11] in the healthy throats of eight out of three hundred and thirty persons in New York who gave no history of direct contact with cases of diphtheria. Only two of these eight persons afterward developed diphtheria. They found nonvirulent, but otherwise characteristic, diphtheria bacilli in twenty-four throats of the same group of persons, and pseudodiphtheria bacilli in twenty-seven.

The pseudodiphtheria bacillus which is occasionally found in the throat is devoid of virulence, and presents certain cultural peculiarities distinguishing it from the genuine diphtheria bacillus. It is not known to be pathogenic. There is much confusion in the use of the term "pseudodiphtheria bacillus," and some writers have described under this name the genuine diphtheria bacillus devoid of virulence. There is probably more than one bacterial species which may be called pseudodiphtherial bacillus.

The mouth and adjacent parts are the most frequent portals of entry of the bacterium of actinomycosis. This organism is likely to lodge near carious teeth.

Although the mouth and throat offer more favorable conditions for the prolonged existence of many microorganisms than do other exposed mucous membrane, nevertheless of the vast hords of microorganisms which must gain access to the mouth only a small number actually persist there. The conditions are evidently unfavorable for the survival of the majority of bacterial species in this situation. Those which are unable to gain a foothold must sooner or later pass down into the stomach or intestine or out through the mouth and nose or perish within the body.

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[11] Welch, "Bacteriological Investigations of Diphtheria in the United States" Am. Journ. of the Med. Sciences, Oct., 1894, n. s., CVIII, 427-461.

[12] Sanarelli, Centralbl. f. Bakter., 1891, Bd. X.

The secretions in the mouth and throat, like most of the fluids of the body, possess some degree of germicidal power. Sanarelli[12] found that the saliva is capable of killing in a short time a moderate number of several pathogenic bacterial species. This was found to be the case with *Staphylococcus aureus, Streptococcus pyogenes, Micrococcus tetragenus, Bacillus typhosus* and *Spirillum cholerae asiaticae*. The diphtheria bacillus, however, survived for twenty-eight to forty days, and *Micrococcus lanceolatus* grew well in saliva, although with rapid loss of virulence.

E. Grawitz and Steffen[13] have confirmed this observation of Sanarelli as regards the pneumococcus, and have found that the virulence of this organism may be restored by cultivating it upon pneumonic sputum prepared as an artificial culture medium. They interpret this as indicating that the virulence of the pneumococcus may be enhanced by certain chemical changes in the composition of the sputum.

There is reason to believe that the virulence of pathogenic bacteria in the mouth, notably of *Micrococcus lanceolatus* and *Streptococcus pyogenes,* may fluctuate, both in the direction of attenuation and of exaltation, but we are not acquainted with the conditions which control these changes.

The salivary glands and ducts are free from bacteria, except near the orifice of the ducts.

Respiratory Passages and Middle Ear.—In normal respiration the bacteria contained in the inspired air must enter the nasal cavities. The anatomical arrangement of the nasal passages renders these an important filtering apparatus for the protection of the deeper air-passages. It has been shown by experiments of Wright that with a respiration of one litre of air per minute from three-fourths to four-fifths of the bacteria of the inspired air are retained in the nasal cavities and their adnexa.

It has already been mentioned that most of the bacteria which enter the conjunctival sac are carried into the nose through the nasal duct. We should therefore expect that the nasal cavities would contain abundant microorganisms.

The bacteria of the healthy nose have been studied by E. Fraenkel, Loewenberg, Hajek, von Besser, Wright, Paulsen, Weibel, Deletti, and others, with much divergence in their results. Some have found few, others many, bacteria in the nose; some have met frequently pathogenic bacteria, others rarely or not at all.

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[13] Grawitz and Steffen, Berliner klin. Wochenschr., 1894, No. 18.

Von Besser[14] describes twelve nonpathogenic species isolated from the noses of thirty persons at work in the laboratory. In the same group of cases he found in the nose *Micrococcus lanceolatus* six times, *Streptococcus pyogenes* five times, *Staphylococcus pyogenes aureus* seven times. In the nasal cavities of twenty-eight convalescent hospital patients he found *Micrococcus lanceolatus* four times, and *Streptococcus pyogenes, Staphylococcus pyogenes aureus,* and the bacillus of Friedländer each once; in twenty-three soldiers and servants, *Micrococcus lanceolatus* four times, *Staphylococcus pyogenes aureus* six times, *Streptococcus pyogenes* once, and the Friedländer bacillus once.

Wright[15] isolated from the nasal secretion of ten healthy persons *Staphylococcus pyogenes albus* six times, the aureus and citreus three times, *Bacillus lactis aërogenes* once, and *Micrococcus tetragenus* once.

Paulsen[16] made sixty-four cultures from the healthy noses of twenty-seven persons. Eleven of these were sterile, nineteen gave ten colonies or less, sixteen gave up to one hundred colonies, twelve several hundred colonies, and six furnished countless colonies. Cocci were found much oftener than bacilli. Only once did he find *Streptococcus pyogenes.* Pathogenic bacteria were not observed in any other of the normal cases. Upon cover-slip preparations he noted spirilla, and Weibel has cultivated a comma bacillus from the nose.

Special interest attaches to the presence of capsulated bacilli in the nose, as these are found with great frequency, if not constantly, in ozaena. The ozaena bacillus of Abel[17] resembles closely the Friedländer bacillus, but is believed by him not to be identical with it. Whether or not this bacillus is ever found in the healthy nose is not established. The Friedländer bacillus and the lanceolate coccus have repeatedly been found in the nose, especially in inflammatory conditions. The rhinoscleroma bacillus is a capsulated bacillus much like the Friedländer bacillus, but differing from it by staining with Gram's method. We do not at present possess an entirely satisfactory differentiation of a group of capsulated bacilli to which Friedländer's bacillus, the ozaena bacillus, the rhinoscleroma bacillus, the capsulated bacillus of Pfeiffer, and some others belong.

The diphtheria bacillus is constantly found in fibrinous rhinitis. Various bacteria, especially diplococci, have been found in the secretion of coryza. When this secretion is abundantly poured out, it often happens that cultures from several drops of it contain very few colonies or are sterile.

[14] Von Besser, Ziegler's Beiträge, Bd. VI.

[15] Wright, New York Med. Journ., July 27, 1889.

[16] Paulsen, Centralbl. f. Bakter., Bd. VIII, p. 344.

[17] Abel, *ibid.*, Bd. XIII, p. 161.

Straus has recently made the important observation that the tubercle bacillus is often present in the nasal cavities of healthy persons who spend much of their time in proximity to tuberculous patients. He examined for tubercle bacilli the contents of the nasal cavities of those engaged about hospital wards containing consumptives. Twenty-nine such examinations were made by the inoculation of guinea pigs. In nine cases the guinea pig developed tuberculosis. Of these nine persons, six were healthy attendants occupied in such work as sweeping the floor and shaking bed linen, one was a patient with a chronic non-tuberculous ailment, and two were medical students who spent several hours daily in the hospital. None of these individuals presented the slightest evidence of tuberculosis. These observations are even more significant than those of Cornet as to the abundance and wide distribution of tubercle bacilli in the neighborhood of consumptives.

Wurtz and Lermoyez have found that the nasal mucus possesses considerable bactericidal capacity.

Von Besser found bacteria abundantly in the larynx and bronchi of human corpses, but he demonstrated that after death the fluids from the mouth and nasopharynx may readily penetrate even into the smaller bronchi, and moreover in his cases the lungs and air passages were diseased; so that he attaches no importance to his observations as bearing upon the question of the presence of bacteria in these parts in health.

Hildebrandt[13] found that culture media inoculated with bits of the lung and of tracheal mucus from recently killed rabbits usually remained sterile, and he concludes that practically all bacteria which enter with the air are retained in the upper air passages and do not penetrate below the larynx. This protection, however, has its limits, as when the inspired air was loaded with fungus spores these could be demonstrated after half an hour in the lungs. That foreign particles in the air may be conveyed into the lungs is evidenced by the coal particles regularly found in the lungs.

Wargunin, in opposition to Hildebrandt. isolated nine different kinds of bacteria from the trachea, bronchi, and lungs of recently killed healthy animals.

The observations of the writer are in harmony with Hildebrandt's results as to the usual absence of bacteria, at least in sufficient number to be demonstrable by ordinary culture methods, in the bronchi and lungs of healthy animals.

At autopsies on human beings bacteria, including *Micrococcus lanceolatus* and *Streptococcus pyogenes,* may be found in the lungs without noticeable lesion of this organ.

[13] Hildebrandt, Ziegler's Beiträge, Bd. II.

The action of the ciliated epithelium and coughing would tend to drive out bacteria which may have entered the trachea and bronchi.

The expired air is free from microorganisms, except as these may be mechanically detached, as in sneezing or coughing.

We are not informed as to the frequency with which bacteria are present in the healthy tympanic cavity. That bacteria may pass up the Eustachian tube into the middle ear is shown by the presence in otitis media of various microorganisms often found in the mouth and nasopharynx, notably *Micrococcus lanceolatus, Streptococcus pyogenes,* the Friedländer bacillus, and the pyogenic staphylococci. There are of course other paths by which microorganisms may be carried into the middle ear, as the lymphatic and blood currents, and in meningitis from the cranial cavity.

Netter found constantly in the middle ear of new born infants, at autopsy, bacteria, and among these were pathogenic forms to which he attributes the frequent occurrence of middle ear inflammations in infants. In autopsies upon one hundred and eight infants less than one year old H. Kossel found otitis media in eighty-five cases. The most common organism in these cases was a delicate bacillus, apparently identical with Pfeiffer's pseudoinfluenza bacillus (38 cases). In addition were found *Diplococcus pneumoniae* (10), streptococci (4), thick bacilli (2), staphylococci (2), and *Bacillus pyocyaneus* (once). One case was tuberculous otitis. Some writers are of the opinion that changes often found in the middle ear of the new born are post mortem alterations.

Stomach and Intestine.—The study of the microorganisms present in the stomach and intestine presents many points of physiological and pathological interest, but we must confine our attention chiefly to those of surgical interest. When we consider the relations of intestinal bacteria to various surgical affections, such as perforative and other forms of peritonitis, appendicitis, and even infections remote from the intestinal canal, it is apparent that this subject claims the attention of the surgeon.

The main sources of the microorganisms of the stomach and intestine are the ingesta and the air. From these sources countless bacteria and fungi of all kinds are introduced into the alimentary canal, but, as is true of other exposed mucous surfaces, only a limited number of species are capable of prolonged existence in this situation.

The meconium of the new born infant is sterile, but within twenty-four hours after birth it usually contains abundant bacteria. Although many varieties of bacteria may be found, Escherich has demonstrated a constant bacterial flora in the faeces of milk-fed infants. These constantly present intestinal bacteria are *Bacillus lactis aerogenes,* predominating in the small intestine, and *Bacillus coli communis,* predominating in the large intestine.

These bacteria remain throughout life as the obligatory and characteristic intestinal bacteria of man in health, and as they are frequent secondary invaders of the body in disease, and may be concerned in various surgical and other diseases, much practical interest attaches to them.

The bacterial flora of the small intestine, particularly in its upper part, is more varied, as tested by plate cultures, than that of the large intestine. In fact, the comparison of the results of microscopical examination and of cultures from the stools indicates that a large number of the bacteria in the faeces are dead.

As there is very little free oxygen in the intestinal canal, the conditions are not favorable for the multiplication of obligatory aerobes. Most of the bacteria found in the intestine are strict anaerobes or facultative anaerobes, but anthrax spores, which require oxygen for their germination, develop into bacilli in the intestinal canal.

It is certain that pathogenic bacteria of many kinds often find their way into the intestinal canal; in fact, there is probably no pathogenic germ which may not in certain individuals and in certain times and places be present in the intestine.

It is true that the acid gastric juice may kill many of the microorganisms which enter the stomach, but there are many which resist its action. If we were to rely exclusively upon the results of experiments in the test tube on the germicidal action of the acid gastric juice, particularly the very acid juice of some animals, we should consider this action a formidable obstacle to the passage of living bacteria into the intestine. But when we consider the insusceptibility of many bacteria to weak acids, the relatively slight and varying acidity of the human gastric juice, the absence at times of any acid in the stomach, the withdrawal in large measure from the direct and concentrated action of the gastric juice of bacteria contained in ingested masses of food and large volumes of fluid, and the rapidity with which the gastric contents may pass into the duodenum, we can understand how microorganisms, even those very susceptible to acids, may find frequent opportunity to enter the intestine. Only a fraction, usually not more than .05 to .1 per cent, of the total acidity of the stomach is due to free hydrochloric acid, and the proteid hydrochlorides possess very little bactericidal power.

Many organisms can grow in the human stomach even when it is very acid, as has been shown by Gillespie.[19] The number and variety of microorganisms which have been isolated from the human stomach by Macfadyen, Abelous, Oppler, Gillespie, and others are very great, but it would serve no useful purpose to enumerate them here. *Bacillus lactis aerogenes, Bacillus coli communis,* and the pyogenic cocci may be specified as having been repeatedly found in the healthy stomach.

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[19] Gillespie, "The Bacteria of the Stomach," The Journal of Pathology and Bacteriology, I, p. 279. Here can be found references to the literature of this subject.

The list of pathogenic bacteria which have been found in the intestinal contents is a long one. Many are present only accidentally and as a transient phenomenon. Some are found with such frequency as to merit special notice. In general, the conditions are not favorable for extensive multiplication of most pathogenic microorganisms in the healthy intestine.

Pyogenic cocci are rarely absent from the intestine. They are often present in such small number as to escape detection, but the great frequency with which these cocci, particularly *Streptococcus pyogenes,* are found in the exudate of perforative peritonitis, where they can rapidly multiply, shows that they are common inhabitants of the intestine. Gessner found a pathogenic streptococcus, probably identical with *Streptococcus pyogenes,* in large numbers in the duodenum of six persons out of eighteen examined at autopsy.

Bacillus pyocyaneus and *Bacillus proteus* are also often present in the intestine. The tetanus bacillus and the bacillus of malignant oedema are regular inhabitants of the intestine of herbivorous animals. *Micrococcus lanceolatus* has been found repeatedly in the human intestine, likewise *Bacillus pyogenes foetidus. Bacillus aerogenes capsulatus* has been found by the writer in the intestine in two cases of perforative peritonitis with production of gas. The occurrence of intestinal actinomycosis and of perityphlitis actinomycotica shows that the actinomyces may find its way into the intestine.

There are various other pathogenic bacteria which have been occasionally found in the intestine, but those which have been mentioned are the ones of chief surgical interest. The specific intestinal infections of typhoid fever, cholera, tuberculosis, and anthrax are not considered here. *Amoeba dysenteriae,* on account of its relation to abscess of the liver and lungs, deserves mention.

In this connection it may be stated that the bile in the normal bile-ducts and gall-bladder is to be regarded as free from bacteria. Under various conditions, however, the colon bacillus and other bacteria, particularly the pyogenic cocci, often wander into the biliary passages. The writer has found very frequently the colon bacillus in cultures made from the interior of gall-stones. It is not uncommon at autopsies to find the colon bacillus, less frequently pyogenic cocci, particularly streptococci, in the bile without any alteration in the bile or evident lesion to explain the migration of these bacteria.

Contrary to an old idea, the bile has not been found to possess any decided germicidal property, at least as regards most bacteria.

Genito-Urinary Tract.—It has been shown by Lustgarten and Mannaberg, Rovsing, Steinschneider with Galewski, Petit and Wassermann, and Hofmeister[20] that the healthy *male urethra* always contains bacteria. These are abundant and varied in the fossa navicularis, and diminish rapidly in number and kind toward the posterior part of the urethra. How far back they extend is not positively known. They were found by Hofmeister as far back as eight centimetres from the external orifice under conditions making it probable that they existed at this point, and were not simply pushed back by the sterilized glass tube which was introduced for the passage of the inoculating wire. Urethral bacteria are usually present in urine voided after sterilization of the meatus and fossa, even in the urine passed toward the end of micturition.

Lustgarten and Mannaberg by microscopical examination of the normal male urethra distinguished ten different kinds of bacteria—namely, four bacilli and six cocci. Among the bacilli was the smegma bacillus, which has already been mentioned in describing the bacteria of the skin (page 442). Among the cocci was a diplococcus which they describe as identical in form and staining reactions with the gonococcus. They isolated in cultures seven species of cocci, white and yellow. Among these cocci was found *Staphylococcus pyogenes aureus*. They observed that the common urethral saprophytes were incapable of causing ammoniacal fermentation of the urine, and that they died when planted in urine.

Rovsing, in opposition to most other investigators, found in the normal urethra pyogenic and non-pyogenic bacteria capable of fermenting urine and identical with many of the bacteria found in cystitis, so that he concludes that the usual source of the bacteria causing cystitis is from the urethral flora and not from the air bacteria.

Steinschneider describes four species of diplococci, identical with forms previously recognized by Lustgarten and Mannaberg, as occurring in the normal urethra. Of these, a milk-white diplococcus, which was met in 87 per cent of the eighty-six healthy and gonorrhoeal cases examined, is regarded as the most common bacterium of the urethra. Next in frequency is an orange-yellow diplococcus found in 44.2 per cent of the cases. These two species are stained by Gram's method, and need not be confounded with the gonococcus. Steinschneider isolated from three cases a grayish white diplococcus, and from one case a lemon-yellow diplococcus, which were decolorized by Gram's stain and which may correspond to Lustgarten's pseudogonococci. He concludes that in only 5 per cent of the cases are diplococci found which are decolorized by Gram.

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[20] Hofmeister, Fortschritte der Medicin, 1893, Nos. 16 and 17. References to other articles cited are contained in this article.

Petit and Wassermann, whose methods were open to objection, cultivated from the normal urethra of four persons five cocci, six bacilli, two sarcinae, and two yeast-fungi. They did not find a pseudogonococcus.

Hofmeister did not find the normal urethral flora as varied as have some observers. Two species of diplococci—viz. a non-liquefying white or yellow, large coccus and a liquefying, smaller gray coccus—he regards as regular inhabitants of the urethra. An orange-yellow and an opaque white liquefying diplococcus he isolated, each from two cases. None of these four diplococci from recent cultures were decolorized by Gram, but he states that he repeatedly found in cover-glass specimens from the urethra diplococci which were decolorized by Gram. Once he found *Staphylococcus aureus*. The normal urethral flora he considers to be non-pathogenic and incapable of surviving long in the urine, although pathogenic bacteria may accidentally be present in the healthy urethra.

The occurrence of the so-called pseudogonococci, as first described by Bumm and Lustgarten and Mannaberg in healthy and diseased urethrae, is of importance in reference to the diagnosis of gonorrhoea by microscopical examination. If all of the well-known points, as to the morphology, staining reactions, and enclosure and arrangement within cells, characterizing the gonococcus be observed, most authorities believe that a mistake in diagnosis is possible only in a very small number of cases, if at all. Undoubtedly a microscopical diagnosis of the gonococcus has been made in some cases upon insufficient evidence, and it must be admitted that a positive microscopical diagnosis is sometimes attended with great difficulties. We now possess methods which enable us without serious practical difficulties to isolate and study the gonococcus in cultures.

It cannot be said that investigations have determined satisfactorily the question as to the relative frequency with which the bacteria of cystitis are derived from the urethra or are introduced from without the body. As *Bacillus coli communis* or *Bacillus lactis aërogenes* is one of the most common bacteria in cystitis, the infection may often be from the intestinal canal through the blood-current by way of the kidneys; some believe more directly by continuity or by the lymphatic stream from the rectum. These bacilli are, however, widely distributed outside of the body, and may be present around the genitals.

Although the results of the bacteriological examination of the healthy urine are contradictory, the weight of evidence is decidedly in favor of the view that the urine in the *bladder* in health is free from bacteria, and that if urethral saprophytes, or even some pathogenic germs, gain entrance to the bladder, they do not long survive in a healthy person.

The *female* urethra contains bacteria, and the anatomical conditions are more favorable than in the male for the passage of the urethral bacteria into the bladder. Von Gawronsky in sixty-two cases examined found bacteria in fifteen—namely, *Streptococcus pyogenes* (3), *Staphylococcus aureus* (8), *Staphylococcus albus* (1), *Bacillus coli communis* (2), *Bacillus tholöeideum* (1). This last bacillus is probably a pathogenic variety of *Bacillus lactis aerogenes.* It is stated that the urethra in these cases was normal.

The *vagina* contains bacteria in varying number and kind. The cervix and uterus are normally free from bacteria. The absence of bacteria from the uterus is not easily explained, but it has its analogy in the absence of bacteria from various other canals and cavities which communicate with passages containing bacteria, as, for example, from the bladder and the bile-ducts. The factors usually cited in explanation of this important provision of nature are mechanical hindrances to the penetration of bacteria resulting from the anatomical structure of the parts and the germicidal properties of the secretions. The principal emphasis is usually laid upon the latter factor.

The bacterial flora of the normal vagina contains various bacilli and cocci, which do not grow on our ordinary culture media, but can be recognized by microscopical examination. Doederlein[21] has described a nonpathogenic bacillus or a group of bacilli which he has cultivated upon a specially prepared acid medium, and which are characterized by marked acid fermentation of sugar. These vaginal bacilli are regarded by Doederlein as the only bacteria to be found regularly in the normal vaginal secretion, and upon their presence or absence he has based his distinction between normal and pathological vaginal secretions. It has, however, been shown by Krönig and Menge that Doederlein's bacillus is often absent from the normal vagina, and that it cannot, therefore, serve as a distinguishing character of the normal secretion.

According to Krönig[22] and Menge[23] the regular bacterial inhabitants of the normal vagina are obligatory anaerobes, and can be cultivated only in anaerobic cultures. They do not describe in detail the characters of these vaginal anaerobic bacilli and cocci, but they say that they are nonpathogenic. In microscopical preparations Menge found in the normal vaginal secretion of nonpregnant women bacilli more abundantly than cocci, but the latter were never missed. He found Doederlein's bacilli in the minority of cases, and more frequently short, thick rods and delicate, thin, curved rods.

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[21] Doederlein, Das Scheidensekret, u. s. w., Leipzig, 1892.

[22] Krönig, Deutsche med. Wochenschrift, Oct. 25, 1894.

[23] Menge, *ibid.*, Nov. 15, 22, 29, 1894.

According to Menge, bacteria capable of cultivation in alkaline aerobic culture media are present abundantly near the outlet of the vagina, but are usually absent, and when present only few, in the upper part of the normal vagina. He found only once out of fifty cases examined a pyogenic coccus. This was *Streptococcus pyogenes,* which existed as a persistent organism throughout the vaginal canal of a nonpregnant woman with gonorrhoea of the cervix and thin cervical discharge. The reaction of the vaginal secretion was alkaline.

Diplococci similar to those from the urethra already described occur also in the vagina, and what was said in that connection as to pseudogonococci applies here. Koplik[24] has isolated from the normal vagina of children a white diplococcus, and from the vaginal discharge in simple, nongonorrheal, vulvovaginitis in children two white diplococci and a yellow diplococcus. None of these were decolorized by Gram's method or need be mistaken for the gonococcus.

The question as to the occurrence of pathogenic organisms in the normal vagina has been and is still a subject of vehement controversy among obstetricians, who are divided into two camps as to the desirability of antiseptic irrigations of the vagina and as to the frequency of so-called autoinfection as a cause of puerperal infections. The question is also of interest to the surgeon in relation to hysterectomy and in general to operations involving the vagina and uterus.

The results of different investigators on this point have been extremely divergent, and, notwithstanding the large amount of work done, it is not possible at present to express a final judgment. Some investigators have found pyogenic bacteria frequently in the vagina of pregnant women. Thus, Steffeck found pus-producing organisms in 41 per cent of the twenty-nine pregnant women examined. Others have failed to find pyogenic bacteria, with the exception of the gonococcus, in all of the cases examined, or have met them only exceptionally. Most of the later investigations in which a proper technique was adopted are in accord as to the infrequency with which pathogenic bacteria, with the exception of the gonococcus, are to be found in the vagina of either pregnant or nonpregnant woman.

In a bacteriological examination of one hundred and ninety-five pregnant women Doederlein found in 55.3 per cent acid normal vaginal secretion, according to his definition. The only bacteria were the acid-producing vaginal bacilli. In 44.6 per cent of the cases he found what he calls pathological secretion, which was feebly acid, neutral, or alkaline, and contained various bacilli and cocci. *Streptococcus pyogenes* he found in 9.2 per cent of the so-called pathological secretions, or in 4.1 per cent of the one hundred and ninety-five cases examined. To the staphylococci present he attributes no importance in the causation of puerperal fever. *Oïdium albicans* is often found in the vagina, but is without significance as a cause of primary infection.

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[24] Koplik, Journal of Cutaneous and Genito-Urinary Diseases, June and July, 1893.

Krönig found the vaginal secretion of all the pregnant women (about five hundred) whom he examined to be acid, the intensity of the reaction varying in different cases. The bacterial flora was of the same general character as that already described for the normal vagina. In no case did he find *Streptococcus pyogenes* or pyogenic staphylococci. His conclusion, that *Streptococcus pyogenes* and the pyogenic staphylococci are never present in the vagina of pregnant women, and that if they should enter they must of necessity be quickly destroyed, cannot be accepted without discrediting the results of other competent investigators, but we may admit that they are present only exceptionally.

Experiments have been made by Krönig and Menge proving that bacteria, including *Streptococcus pyogenes* and pyogenic staphylococci, introduced, even in large number, into the vagina of pregnant or nonpregnant women, disappear in a short time. Thus, Krönig found that *Bacillus pyocyaneus* disappear from the vagina of pregnant women in from ten to thirty hours, the staphylococci in from six to thirty-six hours, and *Streptococcus pyogenes*, with which only three experiments were made, within six hours. No difference was observed, as to the rapidity with which these foreign organisms disappeared, between the normal and pathological secretions in Doederlein's sense, including those containing gonococci. Menge found in nonpregnant women that *Bacillus pyocyaneus* disappeared from the vagina on the average in twenty-one hours, *Staphylococcus pyogenes aureus* in twenty-six hours, and *Streptococcus pyogenes* in twenty-two hours. In only one case did the vagina become infected, and this was with *Staphylococcus aureus* in consequence of an accidental injury to the wall. In the other cases the bacteria immediately after their introduction began to disappear. In most instances an increase in the leucocytes in the vagina was observed.

Menge has attempted to discover the factors concerned in the production of this powerful bactericidal property of the vaginal secretion. He attaches some importance to the acid reaction to which Doederlein had called attention as the essential factor, but this cannot be the sole or the principal factor, as the vaginal secretion of nonpregnant women, although usually acid, may be alkaline, and the bacteria disappeared about as rapidly from the alkaline as from the acid secretion. We cannot at present give any satisfactory explanation of the bactericidal properties of the vaginal secretion.

Irrigation of the vagina with water or with antiseptic diminished the rapidity with which bacteria introduced into the vagina disappeared.

Bacteria introduced into the cervix uteri likewise disappeared in a short time. The secretion here is alkaline.

As a result of the investigations of numerous observers we may conclude that the normal vagina of pregnant and nonpregnant women may contain pathogenic bacteria. With the exception of the gonococcus, such bacteria are not often present, and the vaginal secretion does not, as a rule, permit the prolonged survival of most of the bacteria, including pathogenic forms, which may enter. Powerful as these normal defences are, they may, however, be overthrown under conditions which we now only imperfectly understand.

Much emphasis has been laid upon the fact that pyogenic cocci occasionally found in the vagina have manifested, with few exceptions, little or no virulence when inoculated into animals. It should, however, be remarked that the same negative result of the animal experiment sometimes follows the inoculation of streptococci cultivated from cases of puerperal infection. The animal experiment is not decisive as to the possibilities of these pyogenic bacteria in man, and it has been proven that pyogenic cocci without virulence for animals may infect human beings.[25]

Bacteria in Woman's Milk.—Serious errors of interpretation of their observations have been made by several investigators of the subject of excretion of bacteria by the milk, by not recognizing the fact that milk obtained from the breast of healthy women regularly contains bacteria. The milk as secreted by the gland is undoubtedly sterile in health, but bacteria derived from the skin are mingled with the milk. These bacteria gain access to the milk in the lacteal ducts within the nipple. By far the most common of these bacteria is *Staphylococcus epidermidis albus,* described by investigators of this subject as *Staphylococcus pyogenes albus.* This coccus is a regular inhabitant of the milk in the lacteal ducts near their outlets. Surprise has been expressed by Honigmann[26] and others that of all the cutaneous bacteria this should be usually the predominant or the only one found in the milk, but it appears to the writer that his investigations as to the behavior of this organism in the skin explains satisfactorily the appearance of this coccus in the milk. Of all the cutaneous bacteria, this is the only one which penetrates regularly into the deeper layers of the epidermis and into the glandular appendages of the skin. Occasionally other bacteria, also derived from the skin, are present in the milk. *Staphylococcus pyogenes aureus* has been found in the milk of healthy women in a number of instances.

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[25] A full review of the bacteriological literature of the subject of puerperal auto-infection up to the date of its publication, together with the report of original observations, will be found in a paper by J. Whitridge Williams in The American Journal of the Medical Sciences, July, 1893.

[26] Honigmann, Zeitschrift für Hygiene, Bd. XIV, p. 207.

It is not known how far up the lacteal ducts this normal bacterial flora extends. It is probable that the mammary gland conforms in this matter to the behavior of other glands whose ducts open upon surfaces containing bacteria, and that bacteria in demonstrable number are found only in the excretory ducts near their outlets.

As the staphylococci normally present in the milk have the property of coagulating milk, it can hardly be that any considerable multiplication of these bacteria occurs in the milk within the lacteal ducts. Fokker observed a limited germicidal effect of fresh milk in some cases, but Honigmann in a larger number of experiments with proper technique was unable to determine any such property, even as regards the typhoid bacillus and the cholera spirilla, which are readily killed by blood serum.

Sources of the Bacteria in Surgical Infections

Internal Sources.—We have learned that pathogenic bacteria, and particularly the pyogenic micrococci, may be present upon the skin and various exposed mucous membranes of healthy human beings. These bacteria are of course derived primarily from external sources, but some of them may live for a long time or indefinitely on exposed surfaces of the body, and are found here so often that it is proper to recognize the patient's own body as one source of the bacteria concerned in surgical infections.

Internal sources of wound infection are in general less commonly operative and less dangerous than the external sources. One explanation of this is that the pyogenic bacteria commonly found in the healthy body are often less virulent than those derived from many external sources. *Streptococcus pyogenes* when cultivated from healthy mucous membranes is often of very slight or no virulence, as tested upon animals, although occasionally highly virulent streptococci are found under these circumstances. The pyogenic staphylococci, and particularly *Staphylococcus albus,* are often of greatly attenuated virulence when isolated from the exposed surfaces of healthy persons. The lanceolate micrococcus is present in a virulent state in the mouths of only about 15 per cent of healthy persons, although it is oftener present in a nonvirulent condition. *Bacillus coli communis* obtained from healthy faeces is usually of very slight pathogenic power.

There are various influences which may be cited to explain the relatively slight virulence of many of the pathogenic bacteria present in the healthy body. Among the most apparent are the struggle for existence with the regular saprophytic flora of the body, the influence of the products of the latter organisms, and especially the antibacterial properties of the fluids, secretions, and living cells of the body. That under circumstances which we at present little understand the bacteria of the body may acquire unwonted virulence seems certain.

Highly virulent staphylococci and streptococci may be present on the exposed surfaces of the healthy body without doing any harm. The protection of the body from infection with the pathogenic bacteria which it often harbors is due not so much to the lowered virulence of these bacteria as to the defences which have been set up against their invasion and to the local and general resistance offered to their growth.

These defences may, however, be overthrown, and under these circumstances pathogenic bacteria originally present on exposed surfaces of the body may cause various local and general diseases. The unsatisfactory term "auto-infection" is sometimes applied to diseases thus produced.

The internal organs and fluids of the healthy body are normally free from bacteria. Bizzozero and Ribbert have found bacteria, often within cells, regularly in the lymph-follicles of the normal rabbit's caecum, and Bizzozero has found spirilla often in the epithelial cells lining the necks of the gastric tubules in dogs. These are the only examples known of the presence of bacteria in the living cells of the perfectly healthy body, and here the bacteria have penetrated only a short distance beneath the free surface.

It is probable that pathogenic as well as other bacteria occasionally get into the tissues and circulation of healthy persons without doing any harm. It is claimed by some that the invasion of bacteria into the healthy body is not uncommon, and this doctrine is called latent microbism by some French writers. The occasional occurrence of suppuration in a simple fracture of bone or in other injured parts remote from an exposed surface is best explained, at least in many cases, by the lodgement of pyogenic bacteria which have been carried from an exposed surface to the part by the blood or lymphatic current, and which presumably would have done no harm without the presence of a *locus minoris resistentiae*. The infrequency of this event, however, indicates that the presence of pathogenic bacteria in the internal organs and fluids of the healthy body is exceptional.

Can a wound become infected by bacteria conveyed to it by the circulating blood? This may happen in individuals already infected, although, even when it is certain that pyogenic cocci are present in the circulating blood, a wound may heal without suppuration. The possibility of the infection of a wound in a previously uninfected person by bacteria conveyed by the blood current cannot be denied, but we possess no stringent proof of such an occurrence, and all experience goes to show that wound infection from this source must be very exceptional, if it occurs at all.

With proper technique the surgeon has, as a rule, little to fear from the bacteria of the patient's skin as a source of infection of operative wounds. The only bacterium of the skin which is not usually destroyed by thorough disinfection of the surface is *Staphylococcus epidermidis albus,* which lies often deeper than can be reached by any of the practicable methods of cutaneous disinfection. This coccus is found with great frequency in wounds treated aseptically or antiseptically, but ordinarily it does not interfere with the process of repair. Under certain circumstances, particularly when foreign bodies are introduced into the wound or when there is strangulated tissue in the wound, it may cause rise of temperature and suppuration. It is the most common cause of stitch abscess. It is often associated with other bacteria in suppuration of external wounds and in inflammations involving the skin. It is identical with, or simply a variety of, *Staphylococcus pyogenes albus,* but for reasons which have been stated the latter name seems inappropriate for this ordinary, harmless skin coccus. This white staphylococcus is a widely distributed organism, and it may enter a wound from without as well as directly from the surface of the body.

The source of *Bacillus pyocyaneus* in wounds is often from the skin of the patient, but this is a widely distributed organism, and it may also enter from without.

The mucous membranes which normally harbor bacteria on their surface cannot be completely disinfected by any practicable methods, although it is possible to reduce the number of bacteria, in some situations more readily than in others. The utility of attempting before operation disinfection of a mucous surface—as, for example, in operations involving the conjunctiva, mouth, rectum, and the lower genito-urinary tract—must be determined by experience, and on this point the views of surgeons are not in harmony.

Bacteria cannot be completely kept out of wounds of exposed mucous membranes. The kinds of bacteria in the patient's body which are likely to enter such wounds and the special conditions relating to the bacterial flora of the different mucous membranes have already been described. Although wounds involving such mucous membranes as those of the mouth and intestine, which contain vast numbers of bacteria, including often pyogenic varieties, often heal satisfactorily, and even by first intention, the surgeon has no such guarantee of securing primary union as in operative wounds through the skin. The fact that these wounds often heal quickly and without suppuration demonstrates that the mere presence of certain bacteria, even in large number, in a wound does not necessarily interfere with healing. It also supports the view already advocated that the pyogenic cocci often present on certain exposed mucous surfaces are usually of weakened virulence. But the main factor is, after all, the vital resistance of the living tissues to bacteria, including many virulent species.

The pathogenic bacteria often present on the exposed surfaces of the healthy body are, however, of surgical interest, not so much as a source of primary wound infection, but rather as the specific agents of infection in various local and general surgical diseases. As examples of local diseases of this category may be cited otitis media, appendicitis, perforative and other forms of peritonitis, some cases of cystitis, and certain abscesses. The pneumonias, localized abscesses, pyaemia, and septicaemia which are such frequent complications of diphtheria and certain general diseases, as typhoid fever and other infectious fevers, are due in many instances to the secondary invasion of pathogenic bacteria, particularly pyogenic cocci, normally present on mucous membranes. It is chiefly from the mouth, tonsils, pharynx, and intestine that these invaders enter the body. The primary disease is often one which is attended by some lesion of the mucous membrane which opens the way for the passage of the microorganism. But it is not enough for the gate to be thrown open. The defenders within must be overcome. Hence it is particularly in conditions where the vital powers of resistance are lowered that we meet with affections caused by the invasion of pyogenic bacteria from the exposed surfaces of the body. That under these conditions, as well as under others, attenuated bacteria of the body may become exalted in virulence is highly probable.

The term " auto-infection " is associated particularly with the discussions on the causation of puerperal fever. The bacteriological data entering into this discussion have been given in describing the bacteria of the vagina. The evidence is contradictory, but on the whole is opposed to recognizing so-called auto-infection as a cause of puerperal fever, save in a small proportion of cases.

The bacteria most often found in cystitis are such as might readily gain entrance at times to the healthy urethra, but in most cases of this disease, as in so many others, an essential etiological factor is some primary damage to the part, which permits the survival and growth of the invading microorganisms.

The most common secondary invader of the body is *Bacillus coli communis.* The first observations of the general invasion of the internal organs of the body by the colon bacillus were made by the writer and reported in May, 1890. We have found in 40 per cent of the autopsies at The Johns Hopkins Hospital the colon bacillus in one or more of the internal organs, those most frequently invaded being the lungs, kidneys, liver, mesenteric glands, and gall-bladder. In the great majority of these cases the colon bacilli were present without any organic lesion which could be referred to them. Intestinal lesions were found in 75 per cent of our post mortem cases of invasion of the colon bacillus. Very little importance is to be attached to the demonstration of this bacillus in internal organs of the body at autopsies in cases without any lesion which can be referred to it.

The fact that the colon bacillus is found with great frequency in internal organs of the body after death from all sorts of causes, and without doing any manifest injury, necessitates caution in the interpretation of cases where its presence is associated with definite lesions. That in some of the cases reported as infections by this bacillus its presence was only that of an accidental, secondary invader seems to the writer certain. There can be no doubt, however, that under certain conditions the colon bacillus may be pathogenic. It is prone to settle in parts already damaged either by some other microorganism or by some noninfectious agent. In forty-eight cases of acute inflammation, of various kinds and situations, in which we found the colon bacillus, it was the sole organism present in only fourteen. In the majority of cases of appendicitis and of perforative peritonitis we have found associated with the colon bacillus other bacteria, and particularly the ordinary pyogenic cocci. There is reason to believe that the highly resistant colon bacillus may survive in an inflamed part after the primary organism which started the trouble has died out or has been crowded out by the invader.

The colon bacillus has been repeatedly observed alone or in combination with other bacteria in wounds. That it may be the primary infectious agent in various surgical and other inflammations in different parts of the body is established by numerous observations.

Bacillus lactis aerogenes, which most investigators no longer attempt to separate sharply from *Bacillus coli communis,* is likewise a common invader from the intestinal tract under conditions similar to those relating to the colon bacillus and with similar pathogenic effects.

Streptococcus pyogenes is a far more important cause of surgical infections than is the colon bacillus. It is more commonly present than other pyogenic cocci in appendicitis, peritonitis, and other inflammations of serous membranes, and in septic infections secondary to other diseases. It is a frequent cause of secondary inflammations in the lungs, serous membranes, and other parts, and of general septicaemia in diphtheria, tuberculosis, scarlet fever, typhoid fever, and other infectious fevers, and also in many noninfectious diseases. The source of these various secondary streptococcus infections is often from the mouth, tonsils, throat, and intestine, parts which in their normal condition often harbor streptococci.

Of other bacteria more or less frequently present on exposed surfaces in health, and concerned in various surgical infections, may be especially mentioned *Staphylococcus aureus* and *albus, Micrococcus lanceolatus, Bacillus pyocyaneus, Micrococcus tetragenus, Bacillus proteus,* and the bacillus of Friedländer.

External Sources.—Bacteria derived from without the body are those chiefly concerned in infections of wounds and most other primary surgical infections. Abundant experience has demonstrated that if all pathogenic bacteria from external sources, as well as those which may happen to be upon the surface of the skin, be kept out of an external wound, no suppuration of the wound occurs.

There are two ways by which external bacteria may enter a wound: one is by contact with objects contaminated with bacteria; the other is by the air. Strictly speaking, air infection is also contact infection, but the distinction implied in the customary use of these terms is a useful one. Experimental and clinical observations have shown conclusively that contact infection is far more dangerous and common than air infection.

The main emphasis of modern surgical technique is laid upon the sterilization of all objects, such as instruments, ligatures, dressings, and the hands of operator and assistants, which are brought into contact with the wound. The principal sources of contact infection of wounds, as enumerated by Park,[27] are the skin and hair, instruments, sponges or their substitutes, suture material, the hands of the surgeon and his assistants, drainage materials, dressing materials, miscellaneous—*e. g.* drops of perspiration, an unclean irrigator-nozzle, the nailbrush, the clothing of the operator or the bystanders, etc. Of these objects the most difficult to disinfect is the skin. We have already considered this subject, so far as it belongs to this article, in describing the bacteria of the skin.

The pyogenic cocci, which are the bacteria concerned in the great majority of cases of wound infection, are ubiquitous, and it would be useless to attempt to specify all the objects upon which they have been found. The source from which these cocci are derived is not, however, a matter of indifference. The pyogenic cocci, and particularly the streptococcus, derived from infected persons, as from a case of erysipelas or peritonitis or septicaemia, are more likely to cause serious infection of wounds than cocci of the same species derived from other sources.

Of other bacteria sometimes concerned in wound infection, *Bacillus coli communis* not only is a constant inhabitant of the normal intestine, but is widely distributed in external nature, and *Bacillus pyocyaneus,* often present on the healthy skin, is likewise a widely distributed organism. *Bacillus pyocyaneus* is a comparatively harmless organism in external wounds, but it is capable of exerting serious pathogenic activity, especially in infections from the intestine. The soil is the natural home of the tetanus bacillus, which is abundant in certain localities and rare in other places. It has been found with especial frequency in garden earth and about stables. It is present very commonly in the faeces of herbivorous animals, as has already been mentioned. Sormani observed that tetanogenic material introduced into the alimentary canal of dogs, which subsequently were prevented from receiving additional tetanus bacilli, could be demonstrated as long as sixteen days after its reception. The bacillus of malignant oedema is likewise a common inhabitant of the soil. In infected localities the anthrax bacillus lives in the soil. In fact, the upper layers of the soil are the great home of bacteria of all sorts, and pyogenic cocci have been found in this situation. The proteus bacillus, which is capable of assuming pathogenic activity, is commonly found in decomposing animal substances.

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[27] Roswell Park, "Wound Infection," American Journal of the Medical Sciences, Nov., 1891.

Flies and other insects may be the carriers of all sorts of infectious agents. Pyogenic staphylococci have been found in the excrements of flies, and even the susceptible cholera spirillum passes unharmed through the alimentary canal of flies.

Certain surgical infections are associated particularly with injuries from definite objects, as, for example, necrogenic warts, containing tubercle bacilli, from cadavers; zoonotic erysipeloid from crabs and other shellfish; oyster-shucker's keratitis; actinomycosis from oats and other grains; anthrax from hides, woolsorting, and other handling of material from infected animals.

It has been demonstrated by La Garde[28] that bullets obtained from previously unopened boxes as sent out by the manufacturer are practically sterile, but that if the bullets become contaminated with pathogenic bacteria, the latter are not destroyed in the discharge and transit of the bullet, and are capable of infecting wounds. He was able to infect rabbits with *Streptococcus pyogenes* and other pathogenic bacteria by firing infected bullets through the ear.

As compared with contact infection, infection of wounds from the air is of minor importance, but surgeons are not agreed as to whether or not the dangers of air infection are so slight under ordinary conditions that they need not be taken into consideration at all. In the early days of antiseptic surgery bacteria of the air were thought to be frequent agents of wound infection and the carbolic spray was introduced by Lister with the intention of destroying them. There followed a period when surgeons considered that the air bacteria could be wholly neglected as a source of traumatic infection, and this is probably still the opinion of the majority of surgeons. At present there is a tendency again to pay more attention to the possibilities of infection from this source, and some surgeons have even gone back to the use of the spray.

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[28] La Garde, New York Medical Journal, Oct. 22, 1892.

Bacteria are always present in the air over the ground and around human habitations, whereas sea air at a considerable distance from land and the air at high altitudes is nearly or quite free from microorganisms. Bacteria do not usually occur in the air as single, detached cells, but rather as clumps attached to particles of dust, so that in a perfectly quiet atmosphere, as in a closed room, these particles containing bacteria rapidly settle upon underlying objects. Bacteria, being thus attached to particles of dust, are readily filtered out from the air by passing it through porous substances, such as cotton wool. The bacteria are carried down by drops of rain, and the air of a room may be freed from floating bacteria by producing an artificial rain by some form of douche or spray apparatus.

Whatever creates dust, such as the entrance or exit of a body of students, and other movements in a room, brings bacteria into the air.

It is a fact of fundamental hygienic importance that fine particles, including bacteria, are not detached from moist surfaces even by strong currents of air. Hence comes the hygienic value of using moist cloths in removing dust and in cleansing a room. Substances containing infected material should not be allowed to dry under conditions in which dust therefrom can be conveyed into the air. In the present era of dry dressings for wounds there is frequent opportunity for the scattering of dust from the discharges dried on the dressings in the removal and subsequent handling of these dressings, unless especial care be taken to prevent this in all cases where pathogenic bacteria may be present.

The number of bacteria in the air varies greatly under different conditions. In general it may be said that living microorganisms are less abundant in the air than was formerly supposed. They cannot multiply in the air, and only those whose vitality is not destroyed by drying can exist in the air. Desiccation may lessen the virulence of pathogenic bacteria without actually destroying them.

What interests us chiefly in this connection is to know whether pathogenic bacteria, and more particularly the pyogenic cocci, occur in the air, and, if so, how frequently and under what conditions.

Pyogenic staphylococci and streptococci have been repeatedly found in the air, although generally only in small number. Obtained from this source, these cocci are often of only slight virulence, but highly virulent staphylococci and streptococci have been isolated from the air. Among those who have isolated pyogenic cocci from the air may be especially mentioned von Eiselsberg, Emmerich, Neumann, Prudden, Ullmann, Haegler, C. Fraenkel. Haegler[29] demonstrated that streptococci may preserve their vitality and power of development for at least thirty-six days, and staphylococci for one hundred days, in pus dried on bandages.

Pyogenic staphylococci and streptococci have been found far more frequently in hospital wards and operating amphitheatres than elsewhere. Haegler found staphylococci and streptococci in the majority of his examinations of the air in the wards and operating rooms of the hospital in Basle, and the number of such cocci present was in general proportionate to the opportunity for entrance into the air of cocci from dry material and to the amount of stirring up of dust by movement in the room. He also detected in a number of instances *Bacillus pyocyaneus* in the air. Streptococci have been found frequently in the air of rooms containing cases of erysipelas.

Haegler found the pyogenic cocci on the hair and coats of surgeons, and in cobwebs in hospital rooms, as well as on other objects. He concludes from his investigations that the danger of infection from the air is greater than is assumed by many surgeons.

Schimmelbusch and some other investigators have found pyogenic cocci very rarely in surgical wards and operating rooms. It is sufficiently apparent that various circumstances, such as the care exercised in the destruction or sterilization of material infected with discharges, the use of disinfectants, the isolation of infected cases, and the observance of strict cleanliness, must influence the results of these examinations of the air of hospitals, and that the danger of air infection may be considerable in one place and reduced to a minimum in others.

Air infection may readily become contact infection by bacteria from the air being deposited upon the hands of the operator or his assistants, upon instruments, dressings, or other objects which are brought into contact with the wound.

Microorganisms which are capable of development only within the living body are called obligatory parasites. A facultative parasite is one whose ordinary mode of life is saprophytic, but which is capable of a parasitic existence, and a facultative saprophyte is one whose ordinary existence is parasitic, but which can grow outside of a living host. These distinctions, however, cannot always be sharply carried out in practice. Although we can cultivate the gonococcus outside of the body in specially prepared artificial media, there is no reason to suppose that it multiplies, or even long survives, in the outer world under ordinary conditions, and immediate contact with the infected person is the principal source of infection. The tubercle bacillus also can be cultivated artificially, but conditions must be exceptional which permit its multiplication outside of the body. Unlike the gonococcus, the tubercle bacillus is capable of prolonged survival outside of the body, and, as is well known, it is a widely distributed organism. The leprosy bacillus has not been cultivated artificially, and it is ranked among the obligatory parasites. Intimate contact with an infected person seems to be the usual source of infection, although opinions are divided as to the mode of transmission of this disease. The microorganism causing hydrophobia is unknown, but it is doubtless an obligatory parasite.

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[29] Haegler, Beiträge zur klinischen Chirurgie, Bd. IX, p. 496.

The bacteria causing anthrax, tetanus, melignant oedema, and actinomycosis are facultative parasites. The pyogenic cocci find the best conditions for their multiplication in the living body or material rich in organic matter, but these ubiquitous bacteria can often find natural opportunity for multiplication outside of the body.

The only bacteria infectious for human beings which are positively known to develop spores are the bacilli of tetanus, anthrax, and malignant oedema, all killed by exposure in a moist condition for a few minutes to boiling temperature. It is generally stated that the tubercle bacillus forms spores, but this is not positively demonstrated. There is still greater doubt as to the formation of spores by the bacilli of glanders, typhoid fever, and leprosy. The tubercle bacillus, the pyogenic cocci, and the typhoid bacillus are among the more resistant bacteria which are not proven to form spores.

Portals of Entry of Bacteria in Surgical Infections

The portals of entry or atria of infection are the skin and the exposed mucous membranes of the respiratory, alimentary, and genito-urinary tract, and wounds of these surfaces. The foetus may become infected either by germinal, or far more frequently by placental, transmission of infectious microorganisms. In our laboratory experiments we rarely imitate the precise conditions of natural infection, but we make frequent use of methods of inoculation which occur only exceptionally or not at all under natural conditions, such as the injection of bacteria directly into the vessels, into the serous cavities, and beneath the skin, and forced inhalations of large numbers of microorganisms.

Most of the bacteria concerned in surgical infections are capable of entrance through any portal and of producing infection in any part of the body, but there are some which are restricted to certain modes of entrance and to certain parts of the body. Examples of the latter group are the bacteria causing gonorrhoea and tetanus.

Let us consider briefly the defensive arrangements which exist normally at the various portals of entry. These are partly mechanical, and due to the anatomical structure of the part. The thick epidermal covering of the skin and orifices of the body is impenetrable to most bacteria. The thick layer of laminated flat epithelium covering the mucous membranes of the mouth, oesophagus, and vagina is a hardly less efficient mechanical protection. The more delicate mucous membranes covered by cylindrical epithelium are so situated as to be less exposed to injury, but even these surfaces do not ordinarily permit the penetration of bacteria without the occurrence of some damage to their integrity. The ciliated epithelium of the respiratory tract drives foreign particles toward the natural outlets. The tortuous arrangement of the upper air passages filters out most of the bacteria which are inhaled. Bacteria which may happen to enter the bladder or uterus or the glandular ducts, such as the salivary, biliary, or pancreatic, which are normally free from bacteria, would be likely to be discharged with the secretions. Obstruction of these ducts predisposes to their infection.

There are certain situations, particularly the tonsils and the lymphatic follicles of the intestine, which, by the delicate nature of their covering, are especially exposed to the invasion of bacteria. These are vulnerable parts, as is shown by the frequency with which primary and secondary infections start from them, but there is reason to believe that the lymphatic tissue in these situations is richly endowed with vital properties hostile to the development of bacteria.

Of equal importance with these mechanical defences are the anti-bacterial properties of the secretions on mucous membranes. These properties depend partly on the chemical reaction (gastric juice, vaginal secretion), partly on the antagonism offered to invaders by the regular bacterial flora of the surface, but mainly upon bactericidal qualities at present little understood as to their cause, but unquestionable as to their existence.

Microorganisms find at the gates of entrance living cells and fluids which in health are capable of destroying many of them, and if they pass these gates, it is usually only to be arrested and destroyed at the nearest lymphatic glands. Nor are these protective agencies limited to the lymphatics or to any particular organs: they are present in the blood and everywhere throughout the living body, although more highly developed in some places than in others. Whether the battle against the invaders be within the cells, as is assumed in the phagocytic theory, or outside of the cells, the weapons of attack must be furnished by the cells. The living body is amply protected in health against all ordinary bacteria which may seek entrance, and the study of the etiology of infectious diseases involves the consideration not only of the characters of the specific agents of infection, but also of the ways in which the natural defences of the body have been overcome. There are infectious microorganisms to which the healthiest and strongest body is able to offer no resistance, and there are other microorganisms which are capable of doing harm only when the vital resistance of the body has been lowered.

Can microorganisms penetrate the intact skin or mucous membranes, or must there always be some pathological change or lesion of continuity of these parts to permit their entrance? This question has been variously answered, but, at least as far as certain membranes and certain microorganisms are concerned, the evidence must be regarded as conclusive for the affirmative answer. It cannot be doubted that the infectious agents causing malaria, relapsing fever, and the eruptive fevers may enter the body without any defect in the skin or mucous membranes, but our concern is not with this class of diseases.

Garré,[30] in 1885, was the first to make a self-sacrificing experiment which has since been repeated with similar result by several others. He rubbed into the skin of his forearm, in the same way as one would rub in an ointment, a large quantity of a virulent culture of *Staphylococcus pyogenes aureus.* The skin was left intact. After six hours a prickling sensation, associated with redness and turgescence, developed, and in a few hours more about twenty pustules, each developing about a lanugo hair, had formed. In the course of four days an extensive carbuncle, which discharged pus through seventeen openings, formed, and the axillary glands were swollen.

The largest number of experiments of this character have been by Wasmuth.[31] The experiment succeeds almost invariably when virulent staphylococci are thoroughly rubbed into the human skin, but fails almost constantly to give a positive result upon the skin of animals (rabbits, guinea pigs). The failure with animals is not due to greater impenetrability of their skin to bacteria, for anthrax bacilli and some other pathogenic bacteria when rubbed into the skin of animals are capable of causing infection. Animals are less susceptible than man to these pyogenic cocci—a fact which should not be lost sight of in drawing inferences applicable to man from experiments upon animals with these organisms. Büdinger has shown that pyogenic cocci nearly devoid of virulence for animals may produce abscesses when rubbed into the human skin.

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[30] Garré, Fortschritte der Medicin, Bd. III, p. 165, 1885.

[31] Wasmuth, Centralblatt für Bacteriologie, Bd. XII, pp. 824 and 846, 1892.

The mere application of the bacteria to the surface of the skin produces no infection either in man or animals The bacteria must be well rubbed in or pressed in to cause infection.

Through what channels do the bacteria penetrate the skin in these experiments? There are three possibilities—namely, through the epidermis into the rete Malpighi, into the sweat-glands, or into the hair-follicles and sebaceous glands. Schimmelbusch, Machnoff, and Wasmuth have demonstrated that they enter by way of the hair-follicles, and the last named author considers that they can enter the intact skin in no other way. He finds a principal support for this view in the fact that he was unable to produce any effect by rubbing the cultures into the skin of the ball of the thumb, where there are no hair-follicles, and in the actual demonstration by himself and previous experimenters of the bacteria in the hair-follicles. This conclusion is in accord with the clinical fact that furuncles form almost exclusively in parts of the skin provided with hairs and sebaceous glands, and often begin as pustules around the hairs.

The objection may be raised that these experiments do not actually prove the possibility of infectious agents penetrating the intact skin, as microscopical lesions may be caused by the rubbing or the pressure. Macroscopical and microscopical examinations, however, failed to show any lesion of the epidermis. Still, a microscopical defect might be difficult to detect, and Schimmelbusch considers it possible that in these experiments a minute breach of continuity may be produced.

As experiments upon rabbits with bacilli of anthrax and of rabbit septicaemia have shown, general as well as local infection may follow the rubbing of certain pathogenic bacteria into the skin. These experiments simply show that certain pathogenic organisms are capable of settling in the healthy skin and causing local infections, which may, however, be the starting point of general infections. They do not show that microorganisms can pass through the healthy skin and thence invade the body without producing any damage at the point of entrance. It is a well-known clinical fact that in wounds this local damage may be very slight and readily overlooked. Indeed, there is a certain antagonism between the extent of the local infection at the point of invasion and the likelihood of general infection.

We have no conclusive experimental evidence that bacteria can enter the circulation through an intact mucous membrane without causing any lesion whatever at their point of entry. There are, however, many examples of pathogenic bacteria which are capable of attacking a healthy mucous membrane and of producing local and general infection through this channel. The lesion of the mucous membrane in some of these cases may be slight and not so readily demonstrable. The gonococcus is certainly capable of infecting an intact mucous membrane. A large number of infectious diseases can be produced by feeding cultures or material containing certain pathogenic bacteria. Examples of such intestinal infections are tuberculosis, anthrax, typhoid fever, Asiatic cholera, hog cholera, and chicken cholera. As has already been mentioned, the points in the alimentary canal most vulnerable to infection are the tonsils and the lymphatic follicles.

As is well known, indifferent foreign particles, such as coal dust, may be taken up from the air cells of the lungs and conveyed by the lymphatic current to the bronchial lymphatic glands, but under normal conditions these particles do not enter the general circulation. It is probable that bacteria may be disposed of in the same way. It is said that some of the bacteria of the septicaemias of animals—as, for example, the bacillus of rabbit septicaemia—may enter the circulation through the lungs without leaving behind any manifest lesion. We have no conclusive evidence that *Micrococcus lanceolatus, Streptococcus pyogenes,* or the other pyogenic bacteria can pass through the intact lungs into the general circulation without causing some inflammation of the lungs.

The lungs possess the power of disposing of many pathogenic bacteria which have been introduced into them. There have been many experiments to determine the possibility of infection with anthrax bacilli by the way of the lungs, and the conclusions drawn from them have been widely divergent. Buchner and his pupils claim to have succeeded, but Baumgarten and his coworkers reached a different conclusion. Gramatschikoff finds that the intratracheal injection of anthrax bacilli or spores is incapable of causing infection if infection through the wound be avoided. The bacilli quickly perished in the lungs, sections showing degenerated bacilli within four to ten hours.

No infection of an intact mucous membrane, either in man or animals, has been produced experimentally by the mere application to the surface of the membrane of the pyogenic cocci, although there is clinical evidence of the possibility of such a mode of infection

The chief surgical interest of the question of the permeability of intact mucous membranes to bacteria relates to the explanation of tuberculosis of internal parts without tubercular lesion at any portal of entry, and to cases of osteomyelitis, internal abscesses, and, in general, the cases of so-called cryptogenetic septicaemia and pyaemia, in which the most careful examination fails to detect any point where the bacteria could have entered.

Most authorities are of the opinion that tubercle bacilli can pass through a mucous surface without leaving any trace behind of their presence at the point of entrance. The main support for this doctrine of the passage of tubercle bacilli through intact mucous membranes is found in the clinical facts. Orth and Wesener, however, claim that occasionally animals fed with tubercle bacilli develop tuberculosis of the mesenteric glands without any tubercular alteration of the intestinal mucous membrane. Baumgarten contests this, and holds that in all cases of experimental tuberculosis there is a tuberculous lesion at the point of entry. He explains the cases of tuberculosis of lymphatic glands, of bone, and of other parts observed not infrequently in man without tuberculosis of the lungs or any exposed surface by assuming that they are all instances of inheritance of tubercle bacilli. This well known doctrine of Baumgarten involves also the assumption that tubercle bacilli may remain for an indefinite time alive within the body in a latent condition.

In the opinion of the writer the evidence is in favor of the view that tubercle bacilli may enter the lymphatic circulation without causing any tuberculous affection at the point of entrance. In many cases, however, where this explanation might at first be thought to be the correct one, careful search will reveal the atrium morbi, not infrequently in the form of a small healed tuberculous focus in the lungs or other exposed part. H. P. Loomis has found tubercle bacilli in the bronchial glands without tuberculous lesion in any part of the body.

It is not possible at present either to prove or to disprove, for man, the possibility of the passage through an intact mucous membrane of the pyogenic cocci, which are the organisms usually concerned in the so-called cryptogenetic pyaemic and septic processes. It is true that the most careful examination in many of these cases fails to reveal the atrium of infection, but who can say that it may not have been overlooked? The more painstaking the search, the more likely is some portal of entry to be found. This portal may be the root of a carious tooth, the middle ear, one of the nasal sinuses, or some other part not usually examined at autopsies. Who can feel sure that in the whole vast extent of the alimentary, respiratory, or genito-urinary mucous membranes some minute lesion, serving as a point of entry, does not exist? Such a lesion may be microscopic in size, perhaps no more than a necrotic epithelial cell. Moreover, we have to consider that the original lesion at the point of entry may have healed without leaving any recognizable trace; nor need this lesion be a recent one. There are instances of fatal septicaemia following mere punctures and scratches of the skin which gave rise to scarcely any local inflammation, and the septicaemia may develop after the point of original injury has healed and been forgotten. Bacteria, including pathogenic species, may remain alive, but quiescent, for weeks and months, in some instances it would seem even for years, within the body, and then, as the result of a trauma or some other cause, their pathogenic energies may be stimulated into activity.

While it is certain that in many instances pathogenic microorganisms may invade an intact mucous membrane and cause local and general infection, and that in some instances the organism may enter the circulation and be transported to distant parts without manifest lesion at the point of entrance, and while the possibility of the latter occurrence cannot be disproven in the case of the pyogenic cocci, nevertheless there can be no doubt that pathological alterations and wounds of the exposed surfaces of the body open the way for the entrance of the pathogenic microorganisms in most surgical infections. For some pathogenic organisms, including pyogenic cocci of exalted virulence—such as may come from a case of puerperal peritonitis, for example—all that is needed is that the parts should be thus opened, and infection is sure to follow their entrance. But we have only to contrast the frequency with which streptococcus and other pyogenic infections complicate or follow the infectious fevers, such as typhoid, characterized by lesions of mucous membranes, or the frequency with which infection follows external wounds in diabetic and dropsical persons, with the rarity of such a sequel to injuries of the same parts in a healthy person, to appreciate the importance of other factors than the mere breach of continuity in an exposed surface in predisposing to local and general infections. We shall consider later these local and predisposing causes of surgical infections.

Interesting experiments have been made by Schimmelbusch, Nissen, Pfuhl, and others as to the rapidity of absorption of bacteria from fresh wounds. They have demonstrated that bacteria are taken up within a very short time, by the lymphatic and blood vessels, from a fresh bleeding wound. Mice inoculated with anthrax bacilli at the tip of the tail died of anthrax in spite of amputation of the tail ten minutes after the inoculation. Nissen found anthrax bacilli in the nearest lymphatic glands within one hour and a half after peripheral inoculation of an extremity. The application to smooth fresh wounds by Schimmelbusch of a moderate quantity of a culture of the anthrax bacillus or of a streptococcus lethal to mice was fatal, notwithstanding an immediate attempt at disinfection of the wound with the strongest antiseptics. Schimmelbusch and Ricker[32] were able to demonstrate in cultures from the internal organs (lung, liver, spleen, kidney) anthrax bacilli in an hour, or even half an hour, after the inoculation of fresh wounds of mice with this organism. They showed that this rapid absorption of bacteria from fresh bleeding wounds applies equally to pathogenic and saprophytic bacteria. In cultures from the internal organs made five minutes after the infection of a fresh wound of a rabbit's thigh with *Bacillus pyocyaneus* they found many colonies of this bacillus. In making the cultures it is necessary to use the whole organ finely divided.

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[32] Schimmelbusch, Deutsche med. Wochenschrift, July 12, 1894.

The significance for the individual of the rapid absorption of bacteria from fresh wounds depends, of course, primarily upon the character of the bacteria. If these in small or moderate number are capable of causing fatal septicaemia, as is the case with virulent anthrax bacilli in highly susceptible animals, the issue is necessarily fatal; if, as is the case with the pyogenic cocci, the bacteria do not readily gain a foothold where they can multiply, their absorption is of little consequence in most cases, and, so far as the ordinary saprophytes are concerned, their absorption from fresh wounds is a matter of indifference.

The period during which this rapid absorption of bacteria from a fresh wound takes place is of short duration. As soon as a coagulum has formed on the surface of the wound and the open mouths of the lymphatic and blood vessels are plugged, the conditions are changed, and fine particles like bacteria are no longer quickly transported into the lymphatic and blood circulation. The surface of a healthy granulating wound offers great resistance to the invasion of bacteria, almost as much as an intact exposed surface of the body. Slight injuries, however, such as probing, the removal of dressings, and other manipulations, may convert a granulating surface into a fresh wound, with the accompanying dangers of infection.

Elimination of Bacteria in the Secretions

In former times it was thought that the body gets rid of infectious agents chiefly by their excretion through the emunctory channels. We now know that microorganisms are destroyed within the body by the living cells and fluids, and that this method of freeing the body from living bacteria is far more efficient and of much greater importance than that of their excretion by the emunctories.

Pathogenic bacteria often appear in the secretions in various infectious diseases, and it is of interest to know under what conditions they are excreted and in what secretions they occur. The mode of conveyance of infectious organisms from an infected individual to others, and the practical measures of prevention, are determined in large measure by the manner of elimination of these organisms from the infected body.

Wyssokowitsch, as the result of a long series of experiments, came to the conclusion that bacteria in the circulation are never discharged from the body through the healthy organs, but escape only through some breach of continuity or other lesion in the excretory membrane. Hence those pathogenic bacteria which cause some damage to the excretory surfaces—and there are many such bacteria—are most likely to appear in the excreta, although bacteria without this capacity may escape through lesions preformed from other causes.

Not all subsequent investigators of this subject have been able to confirm this law of Wyssokowitsch, and it is true that bacteria may be eliminated from the circulation by way of the excretions without demonstrable lesion of the organ through which they have passed. It is to be remembered, however, that the lesion may be difficult to detect, or may be of some such undemonstrable character as that of the vascular walls in inflammation which permits the passage of leucocytes and red blood corpuscles.

There have been many special and incidental investigations of the subject of the escape of bacteria with the secretions. Of the experimental investigations relating to the subject in general the most important are those of Wyssokowitsch, Pernice and Scagliosi, and Sherrington.[33]

Sherrington experimented on mice, rabbits, and guinea pigs by the subcutaneous or intravenous inoculation of eleven, mostly pathogenic, species of bacteria. In sixty-eight observations the presence of the specific microorganism introduced was detected in the urine twenty-one times; on eight of these twenty-one occasions the presence of blood in the urine was ascertained by the spectroscope; and in a ninth case gross lesions (tubercle) were found in the kidney. Sometimes the urine contained much coagulable albumin. Of the bacteria of surgical importance tested, *Staphylococcus aureus, Bacillus pyocyaneus, Bacillus anthracis,* and a bacillus probably identical with the Friedländer bacillus were found frequently in the urine. In forty-nine experiments the specific bacilli was found in the bile in eighteen. The bacillus of mouse septicaemia appeared in the conjunctiva, which became inflamed, under conditions making it probable that it did not enter from without. Among the points especially emphasized by Sherrington are the following:

> "At the time when every drop of the circulating blood is teeming with microorganisms there may not be the slightest transit of them into the urinary and biliary fluids then secreted, and they may be completely absent from the aqueous humor of the eye-ball."
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> "When certain pathogenic species are employed, a number, often very considerable, of the injected bacteria tend after a time to appear in the

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[33] Sherrington, "Experiments on the Escape of Bacteria with the Secretions," The Journal of Pathology and Bacteriology, Feb., 1893. This valuable article contains a full review of the literature.

secretions of the kidney and liver, and their escape in the secreta is sometimes accompanied by an escape of actual blood, although not infrequently there is no blood in the secreta."

"The evidence is against believing that when this transit of bacteria across the secreting membrane occurs the membrane is still normal in condition, although at the same time it need not be ruptured or pervious to red blood corpuscles."

"The fact that the escape of the bacteria tends to occur, not immediately upon the introduction of them wholesale into the circulation, but in the late stages of the communicated disease, suggests that the healthy secreting membranes are not pervious to bacteria, and that only after soluble poisons produced by the infection have had time to act upon them do the membranes become pervious to the germs. The fact that species which are innocuous did not in the experiments appear in the secreta at any time is in conformity with these conclusions."

Pernice and Scagliosi experimented with *Staphylococcus aureus, Bacillus prodigiosus, Bacillus anthracis, Bacillus pyocyaneus,* and *Bacillus subtilis,* and they found that these bacteria were constantly excreted through the urine and bile, and might escape through various mucous membranes. This excretion begins six to eight hours after their introduction. Virulent bacteria retain their virulence, as a rule, in the excreta.

There are numerous observations in human beings of the escape of infectious bacteria through the excretions. The pyogenic cocci are particularly prone to settle in the kidney and cause focal inflammations, but even without actual foci of suppuration they often escape into the urine. Nannotti and Baciochi, both in grave suppurative processes and in slight ones, found the specific bacteria with great frequency and of customary virulence in the urine. They disappeared from the urine twenty-four to thirty-six hours after the evacuation of the pus. In experimental pyocyaneus infections the specific bacillus is found regularly in the urine. *Micrococcus lanceolatus* in pneumonia and other pneumococcus infections, the typhoid bacillus in typhoid fever, the streptococci in erysipelas and other streptococcus infections, are often present in the urine. *Bacillus coli communis,* the most common of all secondary invaders, often escapes through the kidney.

Pathogenic bacteria are eliminated very often through the bile. One of the most common lesions in various infectious diseases is the presence of focal necroses, sometimes visible to the naked eye and sometimes seen only with the microscope, in the liver, and these permit the passage of bacteria into the bile. It is not, however, necessary that such necroses should be present in order to permit the escape of bacteria into the bile.

Blachstein, in experiments made under the direction of the writer, demonstrated that the colon bacillus and the typhoid bacillus injected into the veins of rabbits often appeared in the bile, where they frequently remained alive and in large number weeks and months after they had disappeared from all of the internal organs. In many of these cases there were focal necroses in the liver. The passage from the circulation into the bile of *Bacillus anthracis* of *Micrococcus lanceolatus, Streptococcus pyogenes,* the pyogenic staphylococci, *Bacillus pyocyaneus* and of other bacteria has been repeatedly demonstrated. Pathogenic bacteria are quite as frequently discharged through the bile as through the urine.

Of course bacteria in the bile will enter the intestine with this secretion, and it is therefore not always easy to determine whether specific pathogenic bacteria found in the intestine in infectious diseases have been discharged through the wall of the intestine or through the liver. Most of the observations recorded concerning the transit of bacteria through the intestinal wall from the circulation have not been made so as to determine whether or not the escape is really through the intestinal mucosa or by way of the bile. Nevertheless, the frequency with which haemorrhages, necroses, inflammations, and ulcers of the intestinal mucous membrane occur in various infectious diseases makes it highly probable that pathogenic bacteria may be eliminated through this channel. Certain it is that the specific infectious bacteria, not only of diseases like typhoid fever, cholera, and tuberculosis, characterized by definite intestinal lesions, but of many other diseases, such as croupous pneumonia, septicaemia, pyaemia, are often found in the faeces.

Tubercle bacilli may be present in the milk of tuberculous cows even when there is no demonstrable tuberculosis of the udder. The statements as to the frequency of this occurrence vary. Ernst demonstrated by the microscope tubercle bacilli in the milk of 28.5 per cent of the thirty-five tuberculous cows examined, and by inoculation experiments in 50 per cent of fourteen cows. Bang by the inoculation test found tubercle bacilli in the milk of only nine out of sixty-three tuberculous cows without mammary tuberculosis. When the udder is tuberculous the bacilli are always in the milk.

Numerous observations have been made to determine whether or not the pyogenic cocci are excreted with the milk in puerperal infections, but since we now know that *Staphylococcus albus* is regularly, and *Staphylococcus aureus* is sometimes, present in the milk of healthy women, the demonstration of these cocci in the milk in cases of puerperal fever has lost much of its diagnostic significance. There is, however, reason to believe that, although the ordinary source of the staphylococci found in the milk is from the skin, they may be excreted through the gland from the blood in pyogenic infections. The presence of streptococci in the milk is more significant of such excretion than that of staphylococci. In several cases of puerperal fever streptococci have been found in the milk, although more frequently they are missed in this secretion under these circumstances. There is evidence that the pyogenic cocci causing mastitis may enter either from without through the lacteal ducts or may have been deposited from the circulating blood. Karlinski claims to have demonstrated that *Staphylococcus aureus* injected into the blood of rabbits may be excreted through the mammary gland.

Foà and Bordoni-Uffreduzzi and Bozzolo have found pneumococci in the milk of pregnant women affected with lobar pneumonia, and the former investigators demonstrated their presence in the milk of pregnant rabbits inoculated with this organism, and noted pneumococcus septicaemia in young rabbits which sucked the breast of their infected mothers.

The typhoid bacillus has been found exceptionally in the milk in cases of typhoid fever. In experimental anthrax the specific bacillus appears in the milk only exceptionally.

Brunner found *Staphylococcus albus,* von Eiselsberg, Preto, and Tizzoni *Staphylococcus aureus,* in the sweat in cases of pyaemia. The detection of staphylococci, particularly of *Staphylococcus albus,* in the human sweat does not necessarily prove that they have been excreted through the sweat-glands from the blood, for even after the most thorough disinfection of the surface of the skin the white staphylococcus has not usually been destroyed in the deeper layers, as has already been explained in describing the bacteria of the skin. Still, it is probable that pyogenic cocci may be eliminated by the sweat-glands, for Brunner has shown that *Staphylococcus aureus* and *Bacillus prodigiosus* injected into the circulation of swine can be demonstrated in the sweat from the snout after administration of pilocarpine. He was also able to detect the anthrax bacillus in the sweat from the paw of an infected cat after stimulation of the sciatic nerve. He reports only three experiments, and it is desirable that more work should be done on this subject, as the frequency with which pathogenic bacteria may appear in the sweat in infectious diseases is of importance, especially with reference to possibilities of contagion and to measures of disinfection.

In Brunner's experiment with *Bacillus prodigiosus* this organism was found also in the saliva, and there are other observations which indicate the possibility of elimination of bacteria through the salivary glands.

Conditions Favoring the Development of Surgical Infections

There are various conditions, relating partly to the microorganisms concerned and partly to the individual receiving the organisms, which are determining factors in causing infection. The factors pertaining to the individual are usually grouped under the vague but indispensable term "predisposition."

Dosage and Virulence of Infecting Bacteria.—In the case of some pathogenic bacteria a single bacterial cell may be capable of infecting a highly susceptible animal, whereas with a less susceptible animal a large number of the same kind of bacteria may be required. In his interesting studies on the relation of the dosage of bacteria to infection Watson Cheyne[34] showed that whereas a single virulent bacillus of rabbit septicaemia was capable of causing fatal septicaemia in the highly susceptible rabbit, in the less susceptible guinea pig the fatal dose was 300,000 bacilli and upward; between 10,000 and 300,000 bacilli produced abscesses, and smaller doses were without effect. In a mouse a single bacillus of mouse septicaemia, and in a guinea pig a single virulent anthrax bacillus, were capable of producing fatal infection, whereas in rabbits and sheep a considerable quantity of the anthrax bacilli was required to kill the animals.

Animals are in general insusceptible to small quantities of the pyogenic cocci, whether introduced subcutaneously or into the circulation. It generally takes several hundred thousand of the cocci to produce local abscesses by intravenous or subcutaneous injection. Man is more susceptible to these cocci.

This question of dosage is largely one of individual or racial susceptibility on the one hand, and of virulence of the microorganisms on the other hand.

The kind of infection produced by some bacteria varies with the dose. It often happens that the inoculation of a very small number of certain pathogenic bacteria produces no effect, a larger number cause only a local abscess, and a still larger number cause fatal septicaemia. This is illustrated by *Micrococcus lanceolatus,* some varieties of *Streptococcus pyogenes,* and other pyogenic cocci.

We are familiar with varying degrees of virulence in the case of a large number of pathogenic bacteria. Influences known to be capable of affecting the vitality and the virulence of bacteria, such as sunlight, desiccation, saprophytic growth, association with other bacteria or their products, are widely operative in nature. The virulence of a microorganism depends probably mainly upon its capacity to form toxic products, as it is by these products that bacteria chiefly produce their pathogenic effects.

Bacteria which enter the body associated with their toxic products are much better adapted to cause infection than when they enter deprived of these products. These toxic substances, by damaging immediately the cells and fluids which protect the body from infection, enable the invader to gain a foothold which it might otherwise not have secured. Vaillard claims that tetanus spores, deprived by heat or by washing of their toxines, are incapable of germinating in the animal body without the aid of accessory causes, such as the presence of other microorganisms, dirt, other foreign substances, necrotic tissue, although the spores themselves are still alive and in suitable media capable of development. If, however, as is usual in our laboratory experiments, the spores or bacilli be inoculated with the toxin, which in most minute doses is of appalling potency, tetanus is sure to develop. In fact, our experimental tetanus is, as a rule, essentially a pure intoxication.

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[34] Watson Cheyne, The British Medical Journal, July 31, 1886.

The varying results which follow the introduction into the body of pyogenic cocci derived from different sources depend doubtless in part upon whether these cocci enter the tissues already equipped with a reserve force of poisonous products or must begin the fight unarmed. The inoculation of infectious material coming directly from the human body, as from a case of puerperal peritonitis, and received perhaps only in a puncture or scratch of the skin, may manifest disastrous effects with which we are not familiar in inoculations with our artificial cultures of the pyogenic cocci obtained from the same source.

Micrococcus lanceolatus, Streptococcus pyogenes, and the pyogenic staphylococci are notable for the wide variations of their virulence. The lanceolate coccus quickly loses its virulence in artificial cultures.

The writer has reported observations as to the varying virulence of *Staphylococcus aureus* cultivated from different sources. Some broth cultures were fatal to rabbits within twenty-four to forty-eight hours when injected into the circulation in the dose of 0.1 c. c., and other cultures injected in the same way produced no apparent effect in the dose of 1.5 c. c. Between these extremes were cultures of all degrees of intermediate virulence. In the majority of cases 0.2 to 0.3 c. c. of fresh bouillon cultures killed the animal within three to seven days after intravenous injection. The variations in virulence of *Streptococcus pyogenes* are even more striking. Pathogenic bacteria, especially pyogenic cocci, obtained from an infected individual are more likely to be highly virulent than when cultivated from other sources. There are, however, many exceptions to this general rule.

In various ways the weakened virulence of pathogenic bacteria may become augmented. Among the most important of those ways with which we are acquainted are repeated passage through the animal body, association with other bacteria or their products, and the addition of certain chemical substances.

The character of the infection varies often with the degree of virulence of the specific microorganism. Bacterial species, which when highly virulent may kill quickly with septicaemia, may, with weakened virulence, require a much longer period to produce the same effects, or may give rise to only local inflammation without general infection. It is especially with bacteria of attenuated virulence that the matter of dosage may be a controlling factor.

The varying virulence of the pyogenic cocci is one of the factors in the explanation of the remarkably diverse effects which these organisms are capable of producing in the animal body. These cocci may under different circumstances cause all grades and kinds of inflammation, serous, fibrinous, suppurative; they may cause localized inflammations and general infections. But there are other circumstances besides the degree of virulence which control these varying results. Equally important, in many cases more important, controlling factors are the manner of invasion of the microorganism and various local and general predisposing conditions.

In fact, there is no definite and constant relation between the character and the severity of an infection produced in man by pyogenic cocci and the virulence of the cocci cultivated from the case. From the gravest septicaemias cocci of slight degrees of virulence may be cultivated, and from a simple epidermal pustule cocci of high virulence, as tested upon animals. There is reason to believe that the virulence of the microorganisms may be modified during the course of an infection, so that we cannot necessarily draw conclusions as to the primary virulence of the organism at the time of its invasion from the virulence noted in cultures from later stages of the disease.

Bacterial Association.—Bacteria are not usually found in the external world under natural conditions in pure culture, but they are mixed together. Mixed infections are common in human beings. In suppurating wounds we usually find more than one bacterial species. It is of importance to learn the relations of these bacterial associations to infection. We are at present only imperfectly informed as to these relations, but we know that they are often of great significance. Only the more salient points which directly bear upon our theme can be considered here.

The association of one bacterial species with another may be without influence upon the development or the properties of either, or one species may be favorable to the growth or enhance the virulence of another, or one species may be antagonistic to another or in various ways modify its characters. Bacteria exert their influence on each other in large part through their chemical products, and it is often possible to bring about modifications of character by exposing one species to the action of the chemical products of another species.

The pathogenic, the chromogenic, the fermentative, and the other vital manifestations of bacteria may be influenced by their combination with each other. *Bacillus pyocyaneus* may lose its power of producing pigment when combined with certain other bacteria (Mühsam and Schimmelbusch). In mixed cultures of certain bacteria chemical products may be formed and fermentations produced which neither of the species in the culture is capable of producing by itself alone (Nencki, Burri and Stutzer). In mixed cultures of two pathogenic bacteria a larger amount of the toxic products of one or both may be formed than either produces when in pure culture (von Schreider).

The effects following the concurrent inoculation of two kinds of bacteria vary. The association may be without influence as regards infection. The result may be that the development of a deadly species of bacterium is checked by the introduction of another species which is relatively harmless. The only striking instance of this is the effect of the inoculation of the streptococcus of erysipelas, the bacillus of Friedländer, and of several other species of bacteria simultaneously with or shortly before or after inoculation with the anthrax bacillus. These bacteria or their products introduced into the body are capable of preventing the developing of the anthrax bacillus and of rescuing the animal from certain death (Emmerich and others). Various explanations of this effect are given by different writers. The effect is probably referable not so much to a direct antagonism of one species to the other as to an augmentation of the antibacterial properties of the cells, which, according to von Dungern and Metschnikoff, is manifested by increased phagocytic activity of the leucocytes. Bacteria in general which cause suppuration may thereby prevent the development of the anthrax bacillus in the focus of suppuration.

More frequently the concurrent inoculation of two species of bacteria increases the danger to the animal from one or both. A combination of two species, each in itself harmless, may prove fatal to the animal (Roger). A bacterium of attenuated virulence may become augmented in virulence by inoculation in combination with another species which need not necessarily be itself pathogenic, or sometimes simply in combination with the chemical products of another species. The following are examples of the exaltation of the virulence or of the pathogenic effects of one kind of bacterium by combined inoculation with another kind or its products: that of *Micrococcus lanceolatus* by combination with *Bacillus proteus vulgaris,* the anthrax bacillus, or the diphtheria bacillus (Monti, Pane, Mühlmann, Mya); *Streptococcus pyogenes* with *Bacillus proteus vulgaris, Bacillus prodigiosus, Bacillus coli communis* (Klein, Monod, and Macaigne); the pyogenic staphylococci with *Bacillus prodigiosus* or its products and other bacterial products (Grawitz and De Bary and others); *Bacillus diphtheriae* with *Streptococcus pyogenes* or its products (Roux and Yersin and others); *Bacillus typhosus,* with *Streptococcus pyogenes, Bacillus coli communis,* and *Bacillus proteus,* or their products (Vincent, Sanarelli); *Bacillus coli communis* with *Bacillus typhosus* and *Streptococcus pyogenes* (Pisenti and Bianchi-Mariotti); the bacillus of symptomatic anthrax with *Bacillus prodigiosus* (Roger). Mention has already been made of the pathological importance of the association of the tetanus bacillus with other bacteria. Of especial interest are the experiments of Prudden demonstrating that the intratracheal injection of cultures of *Streptococcus pyogenes* in tuberculous rabbits leads to the formation of regular phthisical cavities in the lungs—a result which is not obtained by the inoculation of either microorganism alone. Bernheim has demonstrated that the diphtheria bacillus grows more luxuriantly and with a larger production of toxin in the filtrate of streptococcus bouillon cultures than in ordinary bouillon.

A most important group of infections, many of them of surgical importance, is furnished by the secondary invasion of pathogenic bacteria in the course of various infectious diseases, such as typhoid fever, tuberculosis, diphtheria, scarlet fever, smallpox, and other exanthematous fevers. *Streptococcus pyogenes* is more frequently the infectious agent in these secondary infections than any other organism, but the other pyogenic cocci are not uncommonly concerned. The primary infection increases susceptibility to these common pyogenic cocci, which often find the way open for their invasion by some lesion of an exposed surface of the body. It is also very probable that the primary infection may bring about conditions which enhance the virulence of the bacteria concerned in the secondary infection. Although it is not necessary to suppose that these common secondary invaders are always derived from those which are present in health upon exposed mucous membranes, nevertheless they doubtless often have this origin. Pyogenic cocci obtained from these secondary infections are usually, although by no means always, more virulent than when cultivated from the healthy mucous membranes.

Of especial frequency and importance among the mixed and secondary infections here in consideration is the association of the tubercle bacillus with *Streptococcus pyogenes,* the typhoid bacillus with pyogenic cocci, and the diphtheria bacillus with streptococci.

The characters of the various mixed infections are probably determined less by a direct influence of one bacterial species or its products upon another, although this factor appears in some cases to be an important one, than by the action exerted by one or both species upon the resisting powers of the fluids and cells of the body.

Immunity and Predisposition.—In no class of infectious diseases is the influence of predisposition as an etiological factor more apparent than in many of the surgical infections. If a wound could be kept entirely free from pathogenic bacteria, it would not suppurate, no matter how favorable in other respects the local and general conditions for infection might be. The occurrence of suppuration in human beings from sterile chemical irritants is so exceptional that we need not consider this form of suppuration here. It is not, however, possible in all, or even in most, cases to keep bacteria wholly out of a wound. The examination of so-called aseptic wounds shows with great frequency the presence of bacteria, and notably of the white skin-coccus, but also sometimes of other pyogenic cocci, including *Staphylococcus aureus.* Although the cocci found under these circumstances are usually of weakened virulence, nevertheless, as shown by Büdinger and others, they are of some virulence, and may be decidedly virulent. The frequency with which pyogenic bacteria enter wounds is by no means expressed by the frequency with which wounds suppurate. Every surgeon knows that in certain persons and in certain conditions of the body a wound is much more likely to suppurate than in others, although the same precautions are taken to guard against the entrance of microorganisms. It is therefore a matter of prime importance, no less of practical than of scientific interest, to learn, so far as we may, what are the conditions which predispose an individual to infection. Unfortunately, we are at present only imperfectly informed as to many of these conditions.

Predisposition is of most importance in the etiology of those infectious diseases which are caused by microorganisms to which the individual or the species is not in the highest degree susceptible. The pyogenic cocci, in general, belong to this group of microorganisms, although they may exist in a condition of such exalted virulence that predisposition becomes a factor of no significance.

The degree of susceptibility to a specific microorganism influences not only the capacity to acquire the infection, but also the course, severity, and character of the infection. In an individual of great susceptibility a microorganism of attenuated virulence may produce effects which in a relatively insusceptible person can be accomplished only by a highly virulent microorganism of the same species.

The astonishing variety of affections which may under different circumstances be caused by pyogenic cocci, from a simple epidermal pustule to the gravest septicaemia and pyaemia, from a serous to a suppurative inflammation, are to be explained in part by variations in the local or general susceptibility of the individual, although, as already explained, other factors, such as the degree of virulence of the organism, the manner of invasion, and the number of organisms introduced, are also important. We possess abundant experimental evidence of the fact that in the relatively insusceptible many pathogenic bacteria remain localized, causing inflammation, whereas the same bacteria in the highly susceptible invade the blood and internal organs, causing general septicaemia.

Immunity.—Our comprehension of the nature and importance of predisposition as a factor in the causation of infectious diseases has been facilitated by the increase of our knowledge concerning the factors upon which immunity from the invasion and multiplication of bacteria depends; but our understanding of the nature of immunity is still most incomplete. It is not necessary for our purposes in this connection to enter into a detailed consideration of the various doctrines of immunity, important as are the results of recent investigations upon this subject. It will suffice to state briefly the more important facts and hypotheses so far as they bear upon the question now under consideration.[35]

We distinguish natural or hereditary immunity from acquired immunity. Immunity may be acquired by recovery from an attack of a specific infectious disease, or may be artificially produced by vaccination with the specific microorganism or its products, or by the injection of the blood serum or other fluids from individuals rendered artificially immune.

It is certain that the various kinds of immunity do not all depend upon the same causes. The leading theories of immunity may be brought into two classes—one which attributes immunity to the direct and active intervention of the living cells of the body, and the other which explains immunity by the properties of the extracellular fluids. In the last analysis these properties of the body fluids must depend upon the activities of cells, so that we must have recourse either directly or indirectly to cellular functions in any adequate explanation of immunity.

The leading representative of the cellular theories is the phagocytic theory, so elaborately and charmingly developed by Metschnikoff. This supposes that immunity depends upon the seizure of invading microorganisms by amoeboid cells, chiefly leucocytes and other mesodermic cells, and the subsequent destruction of these organisms in the interior of the cells. According to this view the leucocytes are charged with the defence of the body and engage in a veritable conflict with the parasites. The significance of inflammation, according to Metschnikoff, is to bring the leucocytes to the seat of danger, to which they are attracted by the positively chemotactic substances furnished by the microorganisms. Immunity is acquired when the phagocytes have gained tolerance of the poisons of the specific microorganism and are no longer repelled by them.

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[35] The following statements concerning immunity are taken chiefly from the fuller article on this subject by the writer in Pepper's Text-Book of the Theory and Practice of Medicine by American Teachers, II, Philadelphia, 1894.

There exists in a very large number of cases unquestionable a parallelism between phagocytosis and immunity, but the action of phagocytosis is by no means always apparent. We know that microorganisms may be destroyed by extracellular agencies as well as within the cells, and it is a fair question whether the microorganisms before their reception by cells have not already been damaged by these other agencies. It is true that Metschnikoff has proven that phagocytes may take up living and virulent bacteria, and that these bacteria may degenerate and die in the interior of cells, but he has not shown that, as a rule, bacteria when taken up by cells have suffered no injury from extracellular agencies. We possess direct observations which prove that bacteria introduced into the body may degenerate and die not only within cells, but also outside of them in the humors.

The humoral theories of natural immunity have been based largely upon the demonstration by Nuttall, Buchner, and others of the bactericidal properties of the blood and other fluids of the body. Buchner has given the name of " alexins," and Hankin that of " defensive proteids," to these bactericidal substances. The bactericidal alexins are believed by Kossell and Vaughan to be nucleins or nucleinic acid. Their action is not exerted equally upon all bacteria, and there is often no parallelism between the presence of this bactericidal property in the blood of the normal animal and the insusceptibility of the animal to a given microorganism. The important observation has been made, however, that as the result of the introduction of certain microorganisms the body fluids may acquire bactericidal properties as regards the organism introduced.

The search for the origin of the alexins has led to the view that they are derived directly from the cells, and particularly from the leucocytes. This has led to a partial reconciliation between the phagocytic and the most prominent humoral theories of immunity. The leucocytes and other cells, are indeed, the defenders of the body against intruding microorganisms. They furnish the alexins, the weapons of attack. Where they accumulate the defensive material is concentrated, but it is not necessary that the bacteria should be actually incorporated in the body of the cells, although the germicidal properties may be more intense within than without the cells. Such is the explanation of natural immunity now adopted by Buchner and many others.

There is a kind of acquired immunity which is not known to have a parallel in forms of natural immunity. This is the so-called antitoxic immunity. Here, as the result of vaccination with the specific microorganism or its products, the blood and fluids of the immunized animal have acquired the property of neutralizing the poison formed by the specific organism from which the individual has been immunized. The principles of antitoxic immunity have been worked out for the toxic infections, tetanus and diphtheria. The same principle doubtless holds good for acquired immunity from some other diseases, but for how large a number we cannot say. A most important characteristic of this antitoxic immunity is that by successive injections of increasing amounts of the poisonous substances into the animal the antitoxic or immunizing power of the fluids can be augmented to an astounding degree. It is in this way that the antitoxic power of the fluids may be rendered sufficiently high to exert curative effects when injected after the reception of the specific microorganism or its products. This so-called serum therapy has thus far been applied with beneficial results only to cases of tetanus and of diphtheria, and, as regards human beings, it is more efficient in diphtheria than in tetanus.

Local Predisposition.—The term "predisposition" is often used in a loose sense to designate all sorts of conditions which increase the chances of infection, or which augment susceptibility to infection, or which influence the localization, duration, character, or severity of infections. A name which is used to refer to conditions belonging to such different categories, having often nothing in common, is of course objectionable, but common usage sanctions the term, and there appears to be no better one under which to include the various points here to be considered.

We distinguish racial and individual predisposition, inherited and acquired predisposition, local and general predisposition.

Instances abound of differences in susceptibility to infectious diseases between different species of animals, and there are differences also between different races of men; but the most important kind of predisposition, as regards those surgical infections which we are here considering, is individual predisposition.

This individual predisposition may be either inherited or acquired. It may pertain only to one part or to certain parts of the body, or it may belong to the body as a whole. We cannot in many instances determine whether the predisposition is local or general, and, indeed, it is often impossible to draw any sharp dividing line between local and general predisposition.

Local predisposition may be limited to one or more of the portals of entry for microorganisms, or it may exist at some point within the body, constituting a so-called *locus minoris resistentiae.*

We shall consider first predisposing causes of infection at the portals of entry. We shall have in view under this heading more particularly causes which act locally, but it is to be understood that general predisposing factors to be described later may produce local predisposition.

Under the heading of "Inflammation Produced by Bacteria" Dr. Councilman in the preceding article has described the influence upon pyogenic infections of many local predisposing factors, such as the character of the tissue invaded, local anaemia, passive hyperaemia, the withdrawal of nerve-impulses from a part, rapidity of absorption, the introduction of chemical bacterial products, the presence of foreign bodies, previous attacks of inflammation; and the reader is referred to Dr. Councilman's article for these points. It has there been made clear that any interference with the integrity of the tissues and of the local blood circulation and lymph circulation is likely to render them more susceptible to pyogenic agents, and to influence unfavorably the character and course of a subsequent inflammation. Wounds through a thick layer of adipose tissue or cicatricial tissue or other poorly vascularized parts are less able to resist the action of pyogenic bacteria than wounds of such vascular parts as the face.

To the predisposing conditions enumerated may be added oedema of the tissues. How important this may be inferred from the frequency with which erysipelas and suppuration follow so slight an injury as puncture of an oedematous scrotum or leg. Büdinger found that pyogenic cocci so weakened in virulence as to produce no effect when inoculated into the normal ear of a rabbit set up local suppuration when inoculated into an ear rendered hyperaemic and oedematus by temporary application of a rubber band around the root of the ear.

The withdrawal of nerve-impulses from a part may increase its liability to infection. The factors here concerned are various, the most apparent ones being anaesthesia, disturbances in the lymphatic and blood circulation, and nutritive changes. The question as to the existence of special trophic nerves, the interference with whose function predisposes the part to infectious inflammations, has been considered by Dr. Councilman. In a personal communication Dr. Weir Mitchell has kindly favored the writer with the following expression of his views on this point: "I think it true that the withdrawal of nerve-impulses from a part favors infection; that the withdrawal of nerve influence with partial failure of circulation in some cases of injury still further favors infection; that there is a condition of traumatic nerve-irritation which, probably by its abnormal influence on nutrition, favors infection. A partial injury of a nerve sets up local neuritis and may bring on ulcers of a peculiar character.[36] It seems to me that if we injure a nerve-supply, the changes in muscular and nerve conditions which immediately take place would favor such chemical changes in the tissues as to make them more or less susceptible to infection."

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[36] Interesting examples of such ulcers are here cited by Dr. Mitchell from the forthcoming book of his son, Dr. John K. Mitchell, on Nerve-Injuries and Their Remote Results.

Abnormalities in the secretions on exposed mucous surfaces and in the glands communicating with them may be a local predisposing cause of infection. Obstruction to the outflow of secretions, and the presence of calculi or foreign bodies in cavities or glandular ducts opening upon exposed surfaces are important predisposing causes of infection, as is exemplified by inflammations of the vermiform appendix, renal pelvis, urinary and gall-bladders, urethra, the biliary, pancreatic, and salivary ducts.

In general, all traumatic and pathological lesions of exposed surfaces of the body, such as wounds, haemorrhages, necroses, waxy degeneration, inflammation, ulcers, stricture, strangulation, perforation, increase in greater or less degree the opportunities for the entrance, lodgement, and multiplication of pyogenic and other microorganisms.

A suppurating surface, however, offers considerable resistance to the growth and invasion into the body of most pathogenic bacteria. Pus is endowed with marked bactericidal properties, both in its corpuscular elements and its fluid constituents, and the opportunities for absorption from a suppurating surface are much less favorable than from a fresh wound. Sestini found that the bacilli of anthrax and of rabbit septicaemia when applied to a suppurating wound of the rabbit's skin produced no infection, although they readily did so when introduced into a fresh wound. The existence of suppuration, however, lowers the general resistance of the body to bacteria.

The various lesions which interrupt the continuity and integrity of the exposed surfaces of the body become most dangerous channels of infection when the general resistance of the body to infectious agents is lowered, as in various infectious fevers and constitutional diseases.

Experiments and clinical observations have been made with reference to the amount of damage to the intestinal coats which is requisite in order to permit the passage of bacteria from the intestinal canal into the peritoneal cavity. Most observers have found the fluid in the sac of a strangulated intestinal hernia free from bacteria in the great majority of cases. Garré found bacteria only once in eight cases; Rovsing did not find them at all in five cases; nor did Ziegler in five cases; Tavel and Lanz obtained a positive result only twice in seventeen cases of intestinal strangulation; in two out of three omental strangulations they found bacteria; Tietze found bacteria in four out of nine cases, with the possibility that in some of the four cases they were accidentally introduced from without. Sanguineous hernial fluids with bloody infiltration of the intestinal coats were observed without the presence of bacteria, and even the fluid in sacs containing necrotic intestine did not always contain bacteria. The presence of fibrinous peritonitis over strangulated intestine does not necessarily involve the presence of bacteria in the exudate, as this form of peritonitis may be caused by the absorption of the chemical products of bacteria from the intestine (chemical peritonitis). Definite relations between the condition of the intestine and the presence of bacteria in the hernial sac were not observed.

For some at present inexplicable reason Boennecken found bacteria in the sacs of all of the eight strangulated hernias which he examined.

Arndt has shown by experiments on rabbits that bacteria may pass through the strangulated intestinal wall without the existence of necrosis of the intestine, and when the condition of the intestine is such that it quickly returns to the noraml state upon relief from the strangulation. The presence of bacteria in the hernial fluid does not necessarily involve the development of peritonitis. The conditions which permit bacteria to wander from the intestine through its wall into the peritoneal cavity have not as yet been made clear. Tietze has shown that the fluid from a hernial sac possesses marked bactericidal power over some intestinal bacteria.[37]

The entrance of the colon bacillus into the circulation is usually, in our experience, associated with some lesion of the intestinal mucosa, although this is not invariably the case. Absorption of pathogenic bacteria, particularly the pyogenic cocci, from the diseased intestinal mucosa is a fruitful source of various infections of internal parts, and many cases regarded as cryptogenetic septico-pyaemia have this origin.

The importance of accessory and predisposing causes of inflammations due to bacteria is nowhere better illustrated than in the etiology of peritonitis, as has been set forth by Dr. Councilman " System. Surg." (Dennis), Phila., 1895 Vol. I, page 180. There are two principal classes of predisposing causes of peritonitis—namely, substances which damage the endothelial cells of the peritoneum, and solid, unabsorbable substances.

Of especial importance to the surgeon is a knowledge of the local and general predisposing causes of the infection of wounds. The general predisposing factors we shall consider later. From what has already been said, it is apparent that while the surgeon should use every effort to keep bacteria out of a wound, he should refrain so far as possible from doing anything which interferes with the power of the fluids and cells to overcome invading microorganisms.

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[37] For the literature and the most important experiments and observations thus far made upon the subject of bacteria in hernial sacs consult Tavel and Lanz, "Ueber die Aetiologie der Peritonitis," Mittheilungen aus Kliniken und med. Instit. d. Schweiz, Basle and Leipzig, 1893; Arndt, "Ueb. d. Durchlässigheit d. Darmwand eingeklemmter Brüche für Mikroorganismen," *ibid.*, 1893; and Tietze, Arch. f. klin. Chirurgie, Bd. 49.

Among the local conditions[38] which have been found favorable to the growth in wounds of bacteria which otherwise might be disposed of by the tissues and animals fluids without harm may be mentioned strangulation of masses of tissue by ligatures; the presence of foreign bodies, such as drainage tubes and coarse ligatures; interference with the circulation and absorption and the vitality of tissues resulting from undue pressure or tension; dead spaces; accumulation of the fluid and solid discharges from a wound, especially when associated with tension; necrosis and degeneration of tissues caused by the contact of strong chemical irritants. If it were within the power of the surgeon to keep bacteria entirely out of wounds or to destroy them without damage to the tissues after they enter, the conditions just mentioned could not by themselves alone cause infection, but this power he does not at present possess. The so-called aseptic wound of the surgeon is not usually aseptic in the bacteriological sense; that is, free from bacteria.

The following objections to the insertion of drainage tubes into wounds have been elsewhere formulated by the writer[39]: *First.* They tend to remove bacteria which may get into a wound from the direct bactericidal influence of the cells and animal juices. *Second.* Bacteria may travel by continuous growth or in other ways down the sides of a drainage tube, and so penetrate into a wound which they otherwise would not enter. We have repeatedly been able to demonstrate this mode of entrance into a wound of the white staphylococcus found so commonly in the epidermis. The danger of leaving any part of a drainage tube exposed to the air is too evident to require mention. *Third.* The changing of dressings necessitated by the presence of drainage tubes increases in proportion to its frequency the changes of accidental infection. *Fourth.* The drainage tube keeps asunder tissues which might otherwise immediately unite. *Fifth.* Its presence as a foreign body is an irritant and increases exudation. *Sixth.* The withdrawal of tubes left any considerable time in wounds breaks up forming granulations, and thus both prolongs the process of repair and opens the way for infection. Granulation tissue is an obstacle to the invasion of pathogenic bacteria from the surface, as has been proven by experiment. *Seventh.* After removal of the tube there is left a tract prone to suppurate and often slow in healing.

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[38] The writer wishes to acknowledge his indebtedness to his colleague, Dr. William S. Halsted of The Johns Hopkins Hospital, for information and numerous suggestions as to the influence of many local and general causes predisposing to the infections of wounds.

[39] Welch, "Some Considerations concerning Antiseptic Surgery," The Maryland Medical Journal, Nov. 14, 1891.

The advantage from the employment of drainage tubes or other drainage material is, of course, the removal of secretions, and this indication becomes an urgent one if the cavity with which the tube communicates is infected and suppurates. In a given case the surgeon must weigh the advantages and the disadvantages and act according to his judgment. The practice of most surgeons at the present time of restricting within much narrower limits than formerly the use of drainage tubes, and of discarding them for all wounds which offer a fair prospect of primary union, is a distinct advance in the technique of antiseptic surgery.

The presence of blood in a wound is not itself to be desired, and modern surgeons justly lay stress upon prompt and careful haemostasis in surgical operations. Blood in a wound is not, however, so dangerous a thing as some have supposed it to be, and where for the obliteration of cavities in a wound the choice lies between a blood-clot and the introduction of foreign substances, or the exercise of undue tension by sutures, or the application of a very large number of sutures, or forcible pressure, it is, as a rule, better to take the chances that the spaces will fill with blood (Halsted). Blood possesses bactericidal properties, and experiments made by the writer and Howard[40] showed that virulent pyogenic staphylococci injected into blood-clots which had been allowed to fill wound-cavities did not multiply and occasioned no suppuration. John Hunter was fond of dwelling upon what he called the vitality of blood, and John Chiene has directed attention anew to similar views. Schede brought prominently to the notice of surgeons the value of the blood-clot in the healing of a certain class of wounds, and Halsted[41] further extended the field of its application. The unintended presence of blood in a wound by occasioning undue tension and in other ways may be a dangerous thing, and is something very different from the purposeful application of the method of healing by so-called organization of blood-coagula. It is of the first importance to prevent so far as possible all tension in a wound.

Undue stress is sometimes laid upon artificial devices to close the so-called dead spaces of a wound. These spaces when not drained, nor stuffed with foreign substances, nor obliterated by sutures or pressure, nor permitted to fill with blood, do not, unless very large, remain empty spaces. They quickly fill with exudations of fluids and cells, and these fluids and cells possess antibacterial properties as well as blood. For cavities with unyielding walls, such as those in bone, the blood-clot method of treatment has been found especially useful.

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[40] Welch, "Conditions underlying the Infection of Wounds," Transactions of the Second Congress of American Physicians and Surgeons, II, 1891.

[41] Halsted, "The Treatment of Wounds with especial reference to the Value of the Blood-clot in the Management of Dead Spaces," The Johns Hopkins Hospital Reports, II, No. 5.

Surgeons are not agreed as to the value of the application of antiseptic fluids and substances to fresh wounds. There has been in recent years, under the name of aseptic surgery, a general tendency to consider them useless or harmful, but some surgeons still advocate the antiseptic irrigation of fresh wounds. The question is one which can be decided only by clinical experience. The experimental data bearing upon the question are the only ones appropriate to consider here.

The principal objections urged against the employment of the application of such disinfectants as corrosive sublimate and carbolic acid to fresh wounds are—*first,* that they accomplish little or nothing in the way of destroying bacteria which may have entered the wound; and, *second,* that they cause necrosis or other impairment of the tissues, and thereby weaken or abolish the antibacterial properties of the tissues, and thus predispose to infection.

The conditions for the destruction of bacteria by chemical disinfectants in the fluids and tissues of the animal body, even when the bacteria are only upon an exposed surface, are most unfavorable as compared with those in experiments with the same agents in test tubes. The experiments of Schimmelbusch, already cited, do not, as he seems to suppose, demonstrate the uselessness of antiseptic applications to wounds in general. He found that the fatal infection of mice with *Bacillus anthracis* and that of rabbits with the bacilli of rabbit septicaemia, introduced in large number into fresh wounds, could not be prevented by the irrigation of the wound with corrosive sublimate (1:1000) or carbolic acid (5 per cent) or other antiseptics. But some of the bacteria thus introduced, as has already been explained, are immediately or very soon absorbed from fresh wounds, and if one bacillus or a very few bacilli of the kind used by Schimmelbusch were thus absorbed, the death of the animal was sure to follow; whereas a similar absorption of a few ordinary pyogenic cocci is usually without significance. It is evident that these experiments are not conclusive as to the influence of antiseptics upon bacteria which remain in the wound, and are not applicable to the ordinary conditions of wound infection during a surgical operation.

Henle[42] in his experiments conformed more closely to conditions of wound infection in man. He found that in wounds of the rabbit's ear inoculated with streptococcus pus the streptococci remained in the wound for six hours, after which the cocci began to occupy the neighboring lymph-spaces. He found that regularly up to the end of the second hour a complete disinfection of the wound with sublimate (1: 1000) or carbolic acid (4 per cent) could be attained, and that even after six hours the disinfection sometimes prevented the development of the disease, and, if not completely successful, rendered the subsequent infection milder than in the inoculated control ear which was not disinfected.

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[42] Henle, Centralblatt für Chirurgie, 1894, No. 30, Beilage.

Löffler has shown by his careful tests of the action of various antiseptic substances upon diphtheric throats that it is possible to destroy the superficial bacteria by antiseptics without serious injury to the tissues. It is possible that disinfectants, without actually killing bacteria, may restrain their power of development or weaken their virulence.

Messner[43] experimented by inoculating fresh wounds of rabbits with pus or with cultures containing pyogenic cocci, which caused progressive phlegmonous inflammation with fatal termination. He found that, with one exception, all of the ten wounds inoculated with the cocci, and then irrigated with sterile salt solution and treated aseptically, suppurated, with the development of progressive phlegmons which killed the animal within two weeks. On the other hand, all of the ten wounds, with one exception, similarly inoculated and treated antiseptically with lysol or 3 per cent carbolic acid solution, healed and the animals survived. Two of the wounds, treated antiseptically healed without suppuration; the remaining eight suppurated, showing that the cocci had not actually been destroyed, but the process remained localized. Pus from the wounds treated aseptically preserved its virulence, whereas pus from the wounds treated antiseptically was devoid of virulence when inoculated into animals.

Hermann and others have shown that if carbolic acid, corrosive sublimate, and various other chemical irritants be injected subcutaneously into the tissues and soon afterward pyogenic microorganisms be injected into the same locality, the formation of an abscess is much more likely to follow than when the bacteria are injected into the healthy tissues. But, as Messner has shown, a similar favoring influence upon the development of suppuration under these circumstances is exerted by the injection of common salt solution into the subcutaneous tissues. These experiments are not applicable to the conditions existing in an open wound, but they confirm clinical observations as to the great danger of introducing into the subcutaneous tissues of man, with a hypodermic syringe, fluids containing pyogenic bacteria.

[43] Messner, *Ibid.*

Unquestionably, the presence of necroses, such as may be produced by strong chemical disinfectants, predisposes to pyogenic infection. In some situations, as in a closed cavity like the peritoneal, the presence of even superficial necroses, which may affect only the endothelial cells, is an important predisposing cause of infection, but only experience can decide whether such slight superficial necrosis or other injury which the ordinary antiseptics may produce in an external wound is in itself an important predisposing factor in the pyogenic infection of wounds, or even if a predisposing factor is not more than counterbalanced by beneficial influences exerted by the application of antiseptics. Messner has reported experiments on rabbits which seem to show that the irrigation of fresh wounds with 3 per cent carbolic acid solution does not lessen the vital resistance of the tissues in a wound to subsequent inoculation with pyogenic cocci.

Experiments upon animals, therefore, favor rather than oppose the antiseptic treatment of wounds, as distinguished from so-called asepsis.

We turn now to the consideration of local predisposition existing at some point within the body, the so-called *locus minoris resistentiae*. After the infectious agents have passed through the portal of entry and entered the general circulation they may find local conditions favoring their lodgement and development. Without such local predisposition they are often incapable of doing any harm.

Injury, inflammation, and other preexisting disease of an internal part are important and common conditions favoring the lodgement and growth of microorganisms. The classical experiment of Chauveau many years ago demonstrated the predisposition of injured internal parts to infection. He twisted off without rupture of the skin one of the testicles of a young ram from its vascular connections (bistournage), and observed that when he had injected shortly before the operation putrid fluid containing microorganisms directly into the circulation the injured testicle became the seat of septic gangrene, while without such injection the testicle became necrotic and was absorbed without becoming infected.

The experiments of Rosenbach, Orth and Wyssokowitch, Prudden, and others have shown that bacteria do not readily become attached to the smooth surface of the heart valves, but that pyogenic cocci readily adhere and set up septic endocarditis when the valves have been previously torn by a sterile probe passed down the carotid artery. The predisposition of injured joints and bones to the settlement of pyogenic cocci and of tubercle bacilli is established both by clinical and experimental observations. Pyogenic cocci often invade foci of disease caused primarily by other organisms, as is exemplified in tuberculous and gonorrhoeal arthritis, actinomycosis, echinococcus cysts, amoebic abscesses of the liver, etc. In some situations, particularly in the kidney and urinary tract, the colon bacillus often settles in preexisting foci of disease. The anaemic and dry condition of the lung induced by pulmonary stenosis favors the development of pulmonary tuberculosis. The hyperaemic and moist condition associated with mitral regurgitation is comparatively unfavorable to such development, although by no means excluding this disease. Pyogenic cocci not infrequently settle in the joint lesions of locomotor ataxia and cause suppuration.

The existence of a diseased or injured part within the body by no means involves, of necessity, the localization therein of infectious processes which may be caused by pathogenic microorganisms in the circulation. The damaged part may be spared and an apparently healthy part attacked. The endeavor to obtain experimentally in animals the localization of suppuration in a fractured bone or other injured or diseased part by pyogenic cocci injected into the circulation often fails, and in the hands of Rinne failed so regularly that he opposes, although without sufficient reason, the whole doctrine of *locus minoris resistentiae*. By a different line of experimentation Gottstein came to the same conclusion as Rinne.

We are undoubtedly able in some cases to explain the localization of an infection by such apparent local predisposing causes as those which have been mentioned, but in many, indeed in the majority of, instances of localized infections of internal parts we are unable to give any satisfactory explanation of the localization. We know that many pathogenic microorganisms have a decided preference for certain organs and tissues. The injection of *Staphylococcus aureus* into the circulation of rabbits does not lead to the formation of abscesses in all parts of the body, although the cocci must be carried by the blood current everywhere. The abscesses are found most frequently in the kidneys and myocardium, sometimes in the muscles elsewhere, and in young rabbits often in the bone-marrow and joints. To say that the tissues in one part of the body offer better conditions for the growth of the microorganisms than in other parts is only another way of saying that the organisms produce disease in one part and not in another. Such phrases offer no real explanation unless the nature of these better conditions can be defined.

Sometimes we can explain the localization by the manner of reception of the virus, the vascular relation of the part to infected areas, the size and number of the capillaries, the velocity of the circulation, and the readiness with which foreign particles are filtered out. We know that fine particles are deposited from the lymphatic current in the lymph glands, and from the blood circulation chiefly in the spleen, marrow of the bones, liver, and lymph glands. More often we are unable to give any adequate explanation of the localization of an infectious process in internal parts of the body.

General Predisposition.—Under this heading we shall consider predisposing causes which act more or less generally upon the whole body. The effect of such causes may be to increase the susceptibility of the whole body or only of particular parts of the body to infection. The factors here concerned are for the most part less tangible than the local causes of predisposition.

Organs and parts of the body may inherit special vulnerability to certain infections. Susceptibility to certain infectious diseases may be manifest in races and families. The negro race is less susceptible to yellow fever than the white. Algerian sheep are in large measure insusceptible to anthrax, which is very fatal to other sheep. Black rats are more resistant than gray, and gray rats more resistant than white to anthrax (Müller).

Age influences predisposition, as regards some infectious diseases favorably, as regards others unfavorably. Wounds in children, as a rule, heal more quickly, and with less danger of suppuration should pyogenic bacteria enter, than in old people. Certain infectious diseases are most common in infancy, others in adolescence or in maturity or in old age. Osteomyelitis is much more common in children than in adults. This has been attributed to the predisposing influence of injuries to which children are more liable. Doubtless injuries enter into the causation as a predisposing factor, but it has been shown experimentally that there is a special susceptibility of the bone-marrow of young growing animals to infection by pyogenic cocci injected into the circulation (Rodet, Colzi, Courmont and Jaboulay, Lannelongue and Achard, Lexer). In laboratory experiments young animals, as a rule, are found to be more susceptible to most pathogenic bacteria than old ones. There is a special insusceptibility of sucklings during the first months of life to certain infectious diseases, such as mumps, measles, scarlet fever. As will be explained later, the embryo possesses remarkable insusceptibility to some infections.

There is no evidence that there is any difference in predisposition to infection between males and females, except as regards infections directly related to sexual functions.

General anaemia, induced by loss of blood, has been shown experimentally to increase susceptibility to infection with various microorganisms, including the pyogenic cocci, Friedländer's bacillus, and the anthrax bacillus (Rodet, Gärtner, Chauveau, and others). Operative and other wounds are more likely to suppurate when there has been much haemorrhage than when the loss of blood is slight. In general, impaired vitality and nutrition of the body may predispose to certain infections, including wound infection. More or less plausible predisposing causes operating in this way are bad and insufficient food, overwork, depressing emotions, exposure to heat or cold, overcrowding, bad air, and, in general, insanitary surroundings and all conditions of misery.

A large number of experiments have been made upon animals to determine the influence of various factors in increasing susceptibility to infection. The results of these experiments are interesting and suggestive in many ways. Some of them evidently correspond to conditions observed in man, but it is to be remembered that without additional evidence we have no right to apply the results directly to human beings, or to any other bacteria or other animals than those experimented with. The more important results of these animal experiments are the following:

Prolonged narcosis may impair resistance to some pathogenic microorganisms. Klein and Coxwell made frogs and rats highly susceptible to anthrax by narcosis with ether and chloroform, and similar results have been obtained with curare, alcohol, chloral, morphine, and upon other animals and with other diseases. In speaking of the effect of anaesthetics in predisposing to wound infection Roswell Park[44] says: "There is good reason to think that chloroform and ether administered for some time may produce such changes in the blood and tissues that vital processes of repair, cell-resistance, and chemotaxis may be so far interfered with as to facilitate subsequent infection."

Feser, Hankin, and Müller found that rats fed on bread are more susceptible to anthrax than those fed on meat. Müller observed the same degree of insusceptibility when the extractive substances from meat were fed.

Canalis and Morpurgo, and Sacchi rendered pigeons highly susceptible to anthrax by hunger, and Bouchard noted the lowering or disappearance of artificial immunity in rabbits from anthrax by starvation. Prolonged abstinence from water was observed by Pernice and Alessi to render relatively insusceptible animals more susceptible to anthrax. Charrin and Roger found in fatigue induced by working a treadwheel a factor which increased the susceptibility of rats to anthrax and symptomatic anthrax.

Various chemical substances introduced into the body may increase susceptibility to certain infections. Gottstein and Mya and Sanarelli have shown that poisons, such as chlorates, pyrogallic acid, pyridin, which destroy red blood corpuscles, may render insusceptible animals highly susceptible to certain pathogenic microorganisms, although haematolysis thus produced in the case of some animals and certain microorganisms did not weaken natural immunity. Bonome found that haematolysis and haemoglobinuria caused by injection of water into the circulation of rabbits lessened the bactericidal power of the blood as regards *Staphylococcus aureus.* By feeding white mice with phloridzin, which produces glycosuria, Leo rendered these animals highly susceptible to glanders, from which normally they are immune.

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[44] Park, *loc. cit.*

The most important class of experiments showing the predisposing influence of certain chemical substances upon infection are those in which various ferments and bacterial products have been used. In considering bacterial association (page 486) attention has already been called to the fact that the chemical products of some bacteria favor the development of infection with other bacteria. The susceptibility of an animal to infection with a specific microorganism can often be increased by the preliminary or simultaneous injection of the products of the organism. It is not only the products of pathogenic bacteria which may thus increase susceptibility, but also those of certain saprophytic varieties, particularly of the ordinary putrefactive *Bacillus proteus.* Nor is it necessary that the bacterial products in order to weaken resistance to infection should cause distinct toxic symptoms. We do not possess any satisfactory experimental evidence that the volatile and stinking products of putrefactive decomposition augment susceptibility to infection.

Park, Bouchard, and others lay much emphasis upon auto-intoxication from absorption of fermentative products in the stomach and intestine as a predisposing cause of infection, particularly with the pyogenic cocci. Neumann and Canon, in order to test the predisposing influence of absorption of fermentative products from the intestine, ligated aseptically the small intestine of rabbits near the ileocaecal valve and injected subcuteneously a streptococcus culture. The animals with intestinal obstruction, with few exceptions, died with streptococcus septicaemia, whereas the streptococci injected into normal rabbits produced only local inflammation at the point of injection. The ligation of the intestine close to the stomach was not followed by generalization of the streptococcus infection. So deadly an operation as ligation of the intestine introduces so many other factors than the possible absorption of poisonous intestinal products that these experiments cannot be considered to prove what they were intended to demonstrate. Extirpation of the kidneys, with subcutaneous injection of streptococci, also leads to general streptococcus infection.

That many acute and chronic diseases lower the resistance of the body to pathogenic microorganisms has repeatedly been mentioned in the course of this article, and is universally admitted. The influence of certain infectious diseases in favoring the development of secondary and mixed infections has been sufficiently referred to.

Diabetes mellitus is well known to increase in a marked degree the susceptibility to infection, particularly with pyogenic cocci and the tubercle bacillus. Among other diseases characterized by lessened resistance to infection may be especially mentioned acute and chronic Bright's disease, arteriosclerosis, cardiac disease, alcoholism, syphilis, rickets, scurvy, leucocythaemia, Hodgkin's disease. All of these diseases are prone to lessen resistance to pyogenic cocci, and especially to *Streptococcus pyogenes.* A localized streptococcus infection is much more likely to become generalized when it develops in persons affected with any of these diseases than when it appears in a previously healthy person. Here also attention may be called to the terminal streptococcus septicaemias occurring in Bright's disease, heart disease, and various chronic diseases. They may not be distinguished by characteristic symptoms during life, and are to be ranked among such events as terminal pneumonia and dysentery. The portal of entry is most frequently the lungs, skin, and intestine.

Of 218 autopsies of chronic Bright's disease, general arteriosclerosis, and chronic heart disease at The Johns Hopkins Hospital, in 154 there were definite infections. Of 32 cases of local streptococcus infection, excluding perforative peritonitis, tabulated from the autopsy records of the hospital by the writer, chronic nephritis, general arteriosclerosis, or chronic heart disease was present in 18. In 11 out of 14 cases of general streptococcus infection one or more of these diseases was present. We have observed several cases of general streptococcus infection in leucocythaemia and Hodgkin's disease.

In a large number of cases we are able to find no explanation of the existence of individual predisposition.

In conceding to predisposition its full importance as a factor in the etiology of surgical infections, we are not to forget that pyogenic cocci occur of such virulence that they can readily overcome the natural resistance of the most insusceptible human being.

Foetal Infection

The embryo may be infected by transmission of the specific microorganism with the ovum or semen. The only example of this germinal infection in which the conditions are thoroughly understood is pebrine of silkworms caused by sporozoa, which have been studied in all stages of transit through the ovum and spermatozoa to the infected offspring.

The only infectious disease of human beings which have been proven to be capable of conveyance to the offspring through the ovum or spermatozoa is syphilis. Congenital syphilis is usually due to germinal infection, and it may come from either a syphilitic father or a syphilitic mother.

Intra-uterine or placental infection of the foetus may occur in many infectious diseases. Although the intact placenta is a perfect physiological filter, which does not permit the passage of inanimate particles from the blood of the mother to that of the foetus, nevertheless pathogenic microorganisms are capable of breaking through this barrier. The conditions which permit the passage of microorganisms from mother to foetus can sometimes be demonstrated in the form of definite lesions of the placenta, either preexisting or caused by the specific microorganisms or their products. Circulatory disturbances, haemorrhages, defects in the epithelium covering the chorionic villi, areas of necrosis, and tubercles in the placenta have been observed with more or less frequency in cases of foetal infection, but often no placental lesion could be detected. In the later stages of pregnancy and during parturition the anatomical and physiological conditions would seem to be more favorable for the escape of microorganisms from the mother's blood to the foetus than in the early stages.

Some microorganisms are much better adapted than others to pass through the placenta from mother to foetus. In animals the bacilli of chicken cholera and of symptomatic anthrax and the pyogenic cocci frequently make this passage. Although there has been much controversy as to anthrax, it is now settled that anthrax bacilli often pass from mother to foetus, but generally in such small numbers and without subsequent multiplication in the foetus as to require special methods and careful search for their detection.

In human beings infection of the foetus in utero has been observed in smallpox, measles, scarlet fever, relapsing fever, syphilis, tuberculosis, croupous pneumonia, typhoid fever, anthrax, and affections caused by pyogenic cocci. In general, such infection is exceptional, but in some diseases it is comparatively common. Surgical importance attaches to the fact that both experiments on animals and clinical observations show that the pyogenic cocci are frequently transmitted to the foetus from an infected mother.

Developed tuberculosis in the new-born infant is extremely rare, although it has been observed, but this rarity does not prove that tubercle bacilli may not often be transmitted from mother to foetus. It takes time and susceptibility for tubercle bacilli to produce recognizable lesions. Tubercle bacilli without developed tuberculosis have been repeatedly found in the foetus of tuberculous mothers. Birch-Hirschfeld detected in a seven-month's foetus, removed by Caesarean section from a mother with acute miliary tuberculosis, tubercle bacilli both by microscopical examination and by inoculation of guinea pigs. There were no tuberculous lesions in the foetus. The failure to find tubercle bacilli in a large number of similar cases can hardly be regarded as proof of their absence, when one considers the difficulty of demontrating tubercle bacilli in small number irregularly distributed through a large mass without any lesion to indicate where they are most likely to be found. Gärtner finds that the transmission of tubercle bacilli from mother to foetus is common in the experimental tuberculosis of mice, rabbits and canary birds.

There is reason to believe that the embryo offers great resistance to the growth of tubercle bacilli. Maffucci has found that tubercle bacilli inoculated into hens' eggs before incubation remained quiescent during the period of embryonic development, but caused death of most of the chicks from tuberculosis in three weeks to four and a half months after birth. We have evidence that in human beings living and virulent tubercle bacilli may remain latent in the body a long time. As already mentioned, the chances of penetration of bacilli into the foetus appear to be more favorable during the later period of pregnancy than earlier. The frequency of tuberculosis increases very rapidly with each succeeding week after birth, until during the second half of the first year and during the second year of life fatal tuberculosis is very common, and then becomes less frequent until after puberty. Infantile tuberculosis is far more commonly situated in internal organs, such as the lymphatic glands, meninges, bones, and joints, without tuberculous lesion on any exposed surface of the body, than is tuberculosis of adults.

These are the arguments which are urged by Baumgarten and others with great force in favor of frequent bacillar heritage. The majority of authorities, however, are reluctant to abandon the older views, and the final settlement of this important question involves great difficulties not likely to be soon overcome.

The portal of entry to the foetus is the umbilical vein, and therefore microorganisms would be carried first to the liver and the right side of the heart. Corresponding to this, we find that lesions of the liver and of the right side of the heart are particularly common in congenital infections. Pyogenic cocci, as already mentioned, break through the placental barrier with comparative ease, and these are the bacteria most often associated with endocarditis, which is more common on the right side of the heart in the foetus and new born than in the adult.

Although the foetus may react to pathogenic microorganisms in the same way as the mother, or even more severely, it possesses a remarkable insusceptibility to some infections, as has been proven experimentally and clinically.

There are several instances in which the specific bacteria of croupous pneumonia and of typhoid fever have been found in the stillborn embryos of mothers affected with these diseases, but there is no satisfactory recorded instance of an infant born with the lesions of typhoid fever or those of lobar pneumonia caused by the pneumococcus. Some bacteria which cause localized infections in the mother may produce in the foetus general septicaemia without localizations.

General Considerations Concerning Pyogenic Bacteria

The pyogenic bacteria play such a predominant rôle in surgical infections, and their pathogenic characters present so many peculiarities, that it is appropriate to consider here the general relations of pyogenic bacteria to surgical infections, although the consideration of the special diseases caused by these bacteria does not fall within the scope of this article.

There are no specific bacteria of suppuration. On the one hand the number of bacteria which under special conditions are capable of causing suppurative inflammation is large, and is not limited to any particular group, and on the other hand the bacteria which are most frequently the cause of suppuration are capable of causing other forms of inflammation and of producing infection without inflammation.

Certain staphylococci and streptococci, however, are found in purulent inflammations in human beings so much more frequently than in other microorganisms that they are the pyogenic bacteria *par excellence,* and are the ones generally understood when the expression " microorganisms of pus " or " pyogenic bacteria " is used without any qualification. These staphylococci and streptococci are endowed with pus-producing properties in larger measure than are other bacteria, and of all their pathogenic effects the production of suppurative inflammation is the most prominent.

The most common pyogenic cocci are *Staphylococcus pyogenes aureus, Streptococcus pyogenes* and *Staphylococcus pyogenes albus,* including *Staphylococcus epidermidis albus.* In the second rank, as regards both frequency and virulence, are *Staphylococcus pyogenes citreus, Staphylococcus cereus albus,* and *Staphylococcus cereus flavus.* More common and far more important than the cocci of the latter group are *Micrococcus lanceolatus* and *Micrococcus gonorrhoeae.* Although these are not always ranked among the pyogenic cocci in the restricted sense, they are genuine pus-producers. *Micrococcus tetragenus septicus* is rarely the sole cause of suppuration. The status of *Micrococcus pyogenes tenuis* is uncertain. It is perhaps identical with *Micrococcus lanceolatus.*

There is a long list of bacilli which have been shown with greater or less certainty to be capable of producing suppurative inflammations in man.

The principal ones are *Bacillus pyogenes foetidus, Bacillus coli communis, Bacillus typhi abdominalis, Bacillus tuberculosis, Bacillus pyocyaneus, Bacillus pneumoniae* of Friedländer, *Bacillus proteus.* Actinomyces belongs to the class of schizomycetes and is a pus-producer.

Several anaerobic bacilli have been found both in pure culture and associated with other bacteria in closed abscesses. Of these may be especially mentioned *Bacillus phlegmones emphysematosae* of E. Fraenkel, which is probably identical with *Bacillus aerogenes capsulatus* previously described by Welch and Nuttall, and which occurs in some phlegmons containing gas.

The list of demonstrated pus-producing bacteria is by no means exhausted by those enumerated. In the experience of the writer bacilli are more common causes of spontaneous abscesses in laboratory animals than cocci. A very large number of bacteria are capable of producing experimental abscesses in animals.

It is fair to say that not all of the bacteria mentioned above are recognized as pyogenic for man by all authorities, and particularly that some do not so regard the typhoid bacillus and the tubercle bacillus; but in the opinion of the writer their pyogenic capacity has been demonstrated.

We have very little definite information as to the underlying conditions which control the pyogenic manifestations of such bacteria as the typhoid bacillus, the tubercle bacillus, and others which do not ordinarily cause purulent inflammations. These conditions seem to pertain partly to the degree of virulence of the microorganism and partly to the local susceptibility of the tissues invaded and the general susceptibility of the individual. We find in experiments upon animals that bacteria of weakened virulence in susceptible animals, or those of usual virulence in insusceptible animals, are prone to produce localized abscesses. In an animal which has been rendered artificially immune from septicaemia caused by certain pathogenic bacteria immunity is not usually produced from the development of local abscesses by the specific organism, in case this is capable of forming abscesses.

Statistical statements as to the relative frequency with which the different pyogenic cocci occur in general in suppurative and septic affections vary according to the class of cases which preponderate. *Staphylococcus aureus* is by far the most common microorganism in furuncles and osteomyelitis, and is common in circumscribed subcutaneous abscesses. *Streptococcus pyogenes* is by far the most common organism in spreading phlegmonous cellulitis, inflammations of serous membranes, puerperal infections, and septicaemia. It is the cause of erysipelas in practically all cases of this disease. It is a frequent cause of all kinds of inflammations of the throat and of bronchopneumonia. *Staphylococcus albus* is often associated with the other pyogenic cocci, especially in inflammations involving the skin. It occurs most frequently in the same general class of cases as *Staphylococcus aureus.* Although capable of causing grave infections, the characters of the inflammations in which the white staphylococcus is found alone are usually mild. Two or more species of pyogenic cocci are often combined.

The relative frequency of occurrence of the pyogenic cocci seems to vary somewhat according to the locality. Levy finds in Strassburg *Staphylococcus albus* more frequently than *Staphylococcus aureus* in all suppurative affections except furuncles, and he was unable to confirm the usual statement that the albus is less virulent than the aureus.

The following table gives the results of the bacteriological examination of 135 ambulatory and operative surgical cases by Dowd[45] in the Vanderbilt Clinic and Cancer Hospital in New York. It affords a good idea of the relative frequency of the different pyogenic cocci in the class of cases which most frequently come to the attention of the surgeon:

	Cellulitis, 51 cases	Infected fresh wounds, 17 cases	Old granulating wounds, 18 cases	Healing wounds, stitches, 5 cases	Furuncles, 7 cases	Abscesses, 37 cases
Streptococcus pyogenes alone	9	3	..	..	..	8
Streptococcus pyogenes predominant	23	3	..	..	..	8
Streptococcus pyogenes relatively few	3	1	6	..	..	1
Staphylococcus pyogenes aureus alone	11	1	1	1	7	6
Staphylococcus pyogenes aureus predominant.	8	2	..	..	..	1
Staphylococcus pyogenes aureus relatively few.	13	3	..	..	..	2
Staphylococcus pyogenes or *epidermidis albus* alone.	1	4	2	4	..	2
Staphylococcus pyogenes or *epidermidis albus* predominant.	..	1	..	..	..	..
Staphylococcus pyogenes or *epidermidis albus* relatively few.	10	5	3	..	..	6
Staphylococcus cereus albus	3	1	2	..	..	1
Staphylococcus citreus	1	..	2	..	..	1
Bacillus pyocyaneus	..	..	1	..	..	3
Bacillus coli communis	..	..	..	..	..	3
Very few growths on agar	..	..	3	..	..	3
No growths on agar	..	..	..	..	..	11
Few undetermined colonies	12	2	5	..	..	5

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[45] Charles N. Dowd, "Some Considerations on Different Types of Exudative Inflammation," Medical Record, New York, Sept. 8, 1894.

The cases from which no growth occurred were tuberculous abscesses and buboes. Eight of the 51 cases of cellulitis showed a persistent tendency to spreading inflammation with undermining of the tissues. In all of these 8 cases streptococci were found. In 2 cases of pyaemia *Staphylococcus aureus* and streptococci were present in the original wound, but only streptococci in the metastases.

It is interesting to contrast with these results of the examination of ambulatory and operative surgical cases the results of the bacteriological examination of post mortem cases. In about 500 autopsies at The Johns Hopkins Hospital there were found in 185 cases the following bacteria:

	Number of cases
Bacillus coli communis	105
Streptococcus pyogenes	62
Staphylococcus pyogenes aureus	43
Micrococcus lanceolatus	29
Staphylococcus pyogenes albus	14
Bacillus typhosus, as the cause of special complications	7
Bacillus pyocyaneus	8
Bacillus proteus	4
Micrococcus tetragenus	2
Staphylococcus pyogenes citreus	1
Pneumobacillus of Friedländer	1
Bacillus pyogenes foetidus	3
Bacillus aërogenes capsulatus	3
Undetermined bacteria	35

The streptococcus cases do not include those in which streptococci were found only in phthisical cavities or adjacent lung. The cases with *Micrococcus lanceolatus* are those in which this organism was found without pneumonia or as the cause of some extrathoracic complication of pneumonia. No cases are included in which the bacteria were found simply on exposed surfaces of the body. Among the undetermined bacteria are several interesting pathogenic forms which could not be positively identified with species already described. Mention has already been made of the frequent invasion of the colon bacillus without any pathogenic manifestations.

The preceding table is inserted not with the intention of analyzing in this article the cases[46] composing it, but to show the great differences between the results of the bacteriological study of living surgical cases and those of the autopsy material of a general hospital, and especially to emphasize the preponderance of streptococcus cases over staphylococcus and all other pyogenic cases in such material.

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[46] The analysis of these cases was the subject of the Middleton Goldsmith lecture by the writer in April, 1894 (Trans. N. Y. Pathological Society for 1894).

All of the affections caused by one species of the pyogenic cocci may be caused by any of the others. For example, *Staphylococcus aureus* may produce spreading phlegmons, inflammations of serous membranes, puerperal infections, general septicaemia as well as *Streptococcus pyogenes,* and *Streptococcus pyogenes* may cause circumscribed abscesses and osteomyelitis as well as the yellow or white staphylococcus. Jordan claims that *Staphylococcus aureus* may cause erysipelas, but Petruschky does not regard his observations on this point as conclusive.

Furthermore, these pyogenic staphylococci and streptococci may cause all kinds of inflammation besides the suppurative. They may, and often do, cause serous, serofibrinous, and fibrinous inflammations of serous membranes. *Streptococcus pyogenes* may cause catarrhal and fibrinous inflammations of mucous membranes. Pyogenic cocci may be the cause of simple inflammatory oedema or serous infiltration of the tissues. They are sometimes found in cutaneous vesicles and blebs containing clear serum. They may be the sole organisms present when the inflammatory exudate is haemorrhagic. They may produce extensive necrosis of the tissues with scarcely any inflammatory exudate. We find the same staphylococci and streptococci in those rarer forms of osteomyelitis which do not suppurate as in the ordinary suppurative form. They are the usual cause of periostitis and ostitis albuminosa, in which the exudate is serous. A serous or serofibrinous inflammation caused by pyogenic cocci may be transformed into a purulent one without the appearance of any new species of microorganism.

We cannot at present give any satisfactory explanation of these diverse effects produced by one and the same bacterial species. We seek to explain these differences usually by referring to such factors as the degree of virulence of the organism, the manner of its invasion, the site of infection, and the condition of the patient. Variations in virulence and the general condition of the patient cannot be the sole explanation, for these pyogenic cocci may in the same individual cause a suppurative inflammation in one part of the body and a serous or a sero-fibrinous inflammation in another part, without any bacteriological difference. We have observed this in some instances of multiple serositis, and Schrank has reported a case in which periostitis albuminosa was associated with suppurative osteomyelitis in the same tibia. The same microorganisms, staphylococci and streptococci, were present in the exudate in both situations. They had produced pus in the bone-marrow and a simple serous exudate between the periosteum and bone. The deciding factor must have been in the tissues, and not in the microorganisms or the general predisposition of the individual, and this same factor, the specific character of the tissues, little as we may comprehend its nature, is doubtless the explanation of their varying reactions to the same infectious agents in many other cases—a point which has been justly emphasized by Kurt Müller.

It might be inferred from what has been said as to the interchangeable effects produced by the different pyogenic cocci that no diagnostic or prognostic importance is to be attached to the determination of the particular species of pyogenic coccus present. If the streptococcus can do everything which the staphylococci can do, if each species can produce mild as well as grave infections, it is argued by some that it is of no practical importance to determine what particular microorganism is present in a given case.

It is more important for the surgeon to understand the general relations of bacteria to traumatic and other pyogenic infections than to become familiar with the special characters of the individual microorganisms which cause these infections, and therefore the principal part of this article has been devoted to a consideration of these general relations. But apart from the interest which pertains to the study of all aspects of disease, even those without evident practical bearings, it would be a mistake to suppose that the bacteriological examination of infectious processes caused by pyogenic cocci is devoid of value for diagnosis, prognosis, or treatment.

There are certain general rules as to the characters of the infections most likely to be produced by the different species of pyogenic cocci, and as to the probability of finding a given species in a certain kind of infection. It would lead too far to attempt to consider here all the different surgical infections with reference to these points. A few illustrative examples may be cited.

The white staphylococcus causes severe local and general infections so infrequently, at least in this country so far as we are informed, that the recognition of its exclusive presence in an inflamed external part of the body justifies the probable conclusion that the inflammation will pursue a mild course and be readily amenable to treatment.

Streptococcus pyogenes may possess all degrees of virulence. Although a streptococcus coming from a case of erysipelas or puerperal fever may not be distinguished botanically from a streptococcus coming from a healthy mucous membrane or caught from the air, it is a very different thing in its possibilities for infection. *Streptococcus pyogenes,* far more frequently than other bacteria, causes spreading phlegmonous inflammations and grave forms of septicaemia. The importance and the frequency of streptococcus septicaemias accompanying tuberculosis, diphtheria, typhoid fever, scarlet fever, the puerperal state, erysipelas, cellulitis, and traumatic infections are probably not even yet sufficiently appreciated by physicians and surgeons, notwithstanding the rapid extension of our knowledge of this subject in recent years. Septicaemias produced by other pyogenic cocci under these circumstances, although they may be of equal severity and similar character, are in a small minority.

In view of the fearful pathogenic possibilities with which streptococci may be endowed, a surgeon cannot regard the presence of these microorganisms in a wound or inflamed part with as little concern as he may the white or even the yellow staphylococcus, although it may be that the particular streptococcus in question is of relatively slight virulence. If his patient have chronic Bright's disease or general arteriosclerosis or chronic cardiac insufficiency, the anxiety of the surgeon will be increased. Inflammations of external parts produced by staphylococci are in general more amenable to treatment and require less vigorous interference than those caused by streptococci. Streptococcus cases are in general more dangerous to other surgical patients in their proximity than staphylococcus cases, and are therefore more likely to require isolation.

The gravest staphylococcus infection with which the surgeon has commonly to deal is acute osteomyelitis. In this particular field *Staphylococcus aureus* takes rank over the streptococcus, although there is no specific microorganism of osteomyelitis any more than there is of suppuration in general. Osteomyelitis may be caused by *Streptococcus pyogenes* and other pyogenic bacteria, but, although these other bacteria not infrequently cause periostitis, they are very rarely the cause of uncomplicated suppurative osteomyelitis.

The especial conditions under which, in cases of infection caused by pyogenic cocci, the cocci appear in the blood in sufficient number to be demonstrable by our methods of examination are far from clear. We find them far more frequently in the blood at autopsies, even very fresh ones, than we are able to do during life. The pyogenic cocci, like most pathogenic bacteria, only exceptionally are able to multiply in the circulating blood of human beings. The greater frequency of their presence in demonstrable number at autopsy may be due in part to their multiplication after death, but this cannot be the sole explanation, as the cocci are found in autopsies made very early after death more frequently than they are found during life. The explanation is probably that during the last hours of life they often find suitable conditions for their multiplication in the blood. A similar phenomenon can often be observed in inoculated animals. All bacteria, including those of the typical septicaemias of animals, such as the bacillus of rabbit septicaemia, of anthrax, *Micrococcus lanceolatus,* injected into the blood quickly disappear from the circulation. The pathogenic forms grow outside of the circulating blood, and often do not make their reappearance in any considerable number in the circulation until shortly before death and after the manifestation of grave constitutional symptoms.

This is true of pneumococcus septicaemia, anthrax, and other typical septicaemias of animals.

In examining the blood of living patients for pyogenic cocci a few drops of blood do not generally suffice, as the cocci are rarely so numerous as to be detected thus. The blood is obtained by wet cups by Petruschky, who has developed a special technique for these examinations. To the demonstrations of the white staphylococcus in blood withdrawn by cutting or pricking the skin, which have been made by a number of investigators, the writer attaches no diagnostic significance, for reasons which have been stated. The detection of streptococci is significant. Petruschky and Canon found streptococci in the blood during life in a number of cases of pyaemia, septicaemia, and localized infection. They were more likely to be present in severe than in the milder cases, but they were occasionally found in cases which recovered, and even in very mild infections. Cocci are found in the blood in cases both with and without metastases.

Doubtless pyogenic cocci often enter the circulation in small number from a localized focus of inflammation caused by them. They are often disposed of without doing any harm by the bactericidal cells and fluids. Their fate, however, is not always one which is so advantageous to the patient. They may lodge and grow in internal parts, causing inflammations of serous membranes, ulcerative endocarditis, and other metastatic inflammations. They may survive, and even multiply, in the blood, with or without the production of metastases. These general infections, particularly those with *Streptococcus pyogenes,* are far more likely to occur with primary localized infections, such as erysipelas, infected wounds, abscesses, in persons with chronic Bright's disease, and other conditions which we have repeatedly specified as predisposing causes, than in previously healthy persons.

The usage has grown up among bacteriologists of limiting the name "septicaemia" to infections characterized by a large multiplication of bacteria in the blood during life. Septicaemia in this bacteriological sense occur in man, but they are not common. Far more common are cases of human septicaemia in which a moderate or small number of pyogenic cocci are found in the blood, but there is no definite relation between the number of bacteria in the blood and the character and gravity of the symptoms. We cannot explain these symptoms nor some of the lesions without assuming that they are referable to the action of toxic products of the bacteria, and that these toxic products may be absorbed from localized foci of inflammation.

There are no specific bacteria of either septicaemia or pyaemia. The same bacteria are found in both diseases.

Bacteria of Surgical Infections

The leading characters, especially the pathogenic, of the bacteria concerned in surgical infections will here be briefly described. Certain rare and unnamed and insufficiently identified bacteria which have been described as occurring in surgical diseases will not be considered.

Staphylococcus pyogenes aureus.—Observed by Ogston (1881), first accurately described by Becker (1883), Rosenbach (1884), and Passet (1885). Appears in the form of spherical cells, averaging 0.8 μ in diameter, which by differential staining often show a diplococcus or biscuit shape. Occurs usually in clumps, also in pairs and short chains. May be found both within and outside of pus-cells in abscesses. Stains readily with aniline dyes, including Gram's stain. Forms an orange-yellow pigment, but only in the presence of free oxygen. Grows at ordinary temperatures, most rapidly at body temperature. Grows on all culture media both with and without free oxygen. Liquefies gelatin rapidly. Coagulates milk; clouds bouillon. May survive in dried pus for one hundred days; is killed in a much shorter time by desiccation when contained in thin media. In the moist condition is killed in ten minutes by a temperature of 58° C. (Sternberg). When dried a higher temperature—90-100° C.—is required to kill it in a short time. In bouillon cultures all of the cocci may be killed in five minutes by sublimate, 1 : 1000, but frequently some survive longer, even up to thirty minutes (Abbott). Killed in a few seconds by 3 per cent carbolic acid solution. Virulence variable. Pathogenic properties for animals and man have already been described. (See pp. 472, 483-484, 500-501, and the preceding section, "General Considerations Concerning Pyogenic Bacteria.")

Staphylococcus pyogenes albus.—Cultivated by Rosenbach from pus in 1884. Differs from the aureus only by absence of pigment. As the pigment may form in aureus cultures very slowly, the cultures should be watched for several days, up to two weeks, before making a diagnosis of the albus. Pathogenic effects similar to those of the aureus, but usually the virulence of the albus is less than that of the aureus.

Staphylococcus epidermidis albus (*Welch*).—Is probably only a variety of *Staphylococcus pyogenes albus.* Usually grows somewhat more slowly; liquefies gelatin and coagulates milk less rapidly. Is of little virulence under ordinary conditions. Is a regular inhabitant of the epidermis, lying deeper than can be reached by disinfection of the surface of the skin. Its behavior has already been described (pp. 438, 461, and 464).

Staphylococcus pyogenes citreus.—Cultivated by Passet from abscesses (1885). Differs from the aureus and albus only by forming lemon-yellow pigment in the presence of free oxygen. Pathogenic effects similar to those of the aureus and albus; often of less virulence than the aureus, but may be highly virulent. Is found less frequently than the preceding staphylococci.

Staphylococci forming pigments intermediate in tint between the aureus and citreus occur, also cocci with the production of only a very faint yellowish color, perhaps produced only after prolonged growth. This has led Lubinski and others to the view that these various pyogenic staphylococci are only physiological varieties of one and the same species.

Staphylococcus cereus albus.—Cultivated from two abscesses by Passet (1885). Pyogenic power not considered to be demonstrated by Passet and Flügge. Levy, however, found this coccus in pure culture in abscesses and other inflammations, and produced suppuration by inoculating it into the rabbit's eye. Resembles the aureus and albus, but it does not liquefy gelatin and it forms no pigment.

Staphylococcus cereus flavus was isolated by Passet in pure culture from a chronic suppurative periostitis, but it does not appear to have been found again in abscesses. Passet was unable to cause abscess by inoculating it into animals. Differs from *Staphylococcus cereus albus* only by the formation of lemon-yellow pigment.

Streptococcus pyogenes; Streptococcus erysipelatos.—Observed by Ogston in pus (1881); cultivated by Fehleisen from erysipelas (1883), by Rosenbach from pus (1884). The streptococcus of erysipelas does not differ in morphology or cultural characters from *Streptococcus pyogenes.* The same pathogenic effects may be produced by each in animals and in man, so that the weight of evidence is in favor of the identity of *Streptococcus erysipelatos* with *Streptococcus pyogenes,* although opinions are still divided on this question.

Streptococcus pyogenes grows in chains of variable length. The individual cocci vary in size, sometimes in the same chain. They average somewhat larger than the staphylococci. In pus they occur in chains, also in pairs. Stain readily with aniline dyes and by Gram's method. Grow with or without free oxygen in all ordinary culture media. Most streptococci grow at room temperature, but more slowly than at body temperature. *Streptococcus pyogenes* does not liquefy gelatin, or does so slightly in some cases. Forms small, gray, granular colonies on gelatin and agar. Grows invisibly on potato, but may grow visibly. May or may not coagulate milk and cloud bouillon. Some cultures are short-lived, others may live several months. *Streptococcus pyogenes* may survive in dried pus for fourteen to thirty-six days. Is killed in ten minutes by exposure to 54° C. (Sternberg). Is killed in eight seconds by 3 per cent carbolic-acid solution. Inoculated cutaneously into the rabbit's ear, virulent streptococci usually produce erysipelas, from which the animal recovers. The virulence is extremely variable, and is best tested by inoculating mice into the peritoneum, either with the first generation of a pure culture or directly with the blood or exudates containing the streptococci. Very virulent streptococci in small doses produce rapidly fatal septicaemia in mice; less virulent ones, a more or less protracted septicaemia or local inflammations; and the mouse may die after a longer or shorter period from the effects of the inoculation without the presence of streptococci at the autopsy. Streptococci are often devoid of virulence for mice. The virulence for mice does not correspond definitely to the character and severity of the streptococcus infection in the patient from whom the streptococci were obtained, although it is a general rule, with many exceptions. that the most virulent streptococci come from grave streptococcus infections in man. The pathogenic properties of streptococci for man have already been mentioned so far as they relate to surgical infections. (See pp. 447, 448, 487, and the preceding section, " General Considerations Concerning Pyogenic Bacteria.")

Streptococci obtained from different sources, and even those cultivated from different cases of the same disease, vary greatly in morphology, cultural characters, and pathogenic properties. So far as virulence is concerned, this property alone cannot serve as a basis of distinction into species, as this is the most variable of all properties of pathogenic microorganisms, and in the case of no microorganism more variable than with *Streptococcus pyogenes.* The virulence of streptococci appears to vary not only in degree, but in kind, so that a streptococcus endowed with the property of producing one kind of infection—as, for example, erysipelas—may not be qualified under ordinary conditions to produce another kind of infection—as, for example, an abscess. But we observe such ready transformations in these varying degrees and qualities of virulence, and such modifications of the pathogenic effects by other circumstances than the virulence, such as the manner and site of invasion and the susceptibility of the individual, that it seems hopeless to attempt any division into physiological varieties on the basis of the quantity and quality of virulence, significant as this property is for our understanding of diverse streptococcus diseases in man.

Many attempts have been made to establish different species or varieties of streptococci on the basis of morphological, cultural, and pathogenic differences. These attempts have met with only partial success. They have tended to demonstrate the great variability of one and the same species, rather than to establish definite and constant distinctions between supposedly different species or varieties. So far, at least, as the streptococci which we ordinarily meet, and particularly those of interest for human pathology, are concerned, most authorities are of the opinion that no satisfactory division into separate species can at present be established, although it is convenient to classify streptococci according to certain prominent but varying characteristics. The characteristics which are most useful in this classification are those which appear in bouillon cultures. They are the length of the chains, the presence or absence of cloudiness of the bouillon, and the kind of sediment produced by the growth of the cocci. We thus distinguish short-chained streptococci (*Streptococcus brevis*), long-chained streptococci (*Streptococcus longus*), streptococci which render bouillon cloudy and those which do not, streptococci which form flocculent or sandy or scaly or viscous sediments. The name *Streptococcus conglomeratus* is given to a streptococcus which grows, without clouding the bouillon, in the form of dense, separate particles, scales, or thin membranes at the bottom and sides of the tube, and on shaking the sediment it breaks up into little specks without producing uniform, diffuse cloudiness. On microscopical examination the chains in the latter case are long and interwoven in conglomerate masses. Streptococcus chains may be straight or wavy or twisted. These various distinctions are only of relative value. One form may change into another. Virulent streptococci may be found among all the groups mentioned. The streptococcus of erysipelas and most of the streptococci from abscesses and septicaemia grow in long chains in bouillon.

Micrococcus tetragenus.—Isolated by Koch and Gaffky (1881) from phthisical cavities. Grows in tetrads enclosed in gelatinous capsules. Stains by Gram. Grows on all media at room temperature, with or without oxygen. Forms elevated, white, non-liquefying colonies on gelatin. Pathogenic for mice, guinea pigs, and, by intravenous and intraperitoneal inoculation, also for rabbits. Found not infrequently in phthisical cavities and sputum, occasionally in association with pyogenic cocci in abscesses connected with carious teeth and about the neck and jaws and middle ear, rarely in abscesses elsewhere. It has been considered to be nonpathogenic for man, but it has been found in pure culture in closed abscesses in man, and Viquerat has proven experimentally that it is capable of causing suppuration in human beings. He considers that suppurations produced by the tetragenus alone are mild in character, painless, with little reaction, easily cured. Boutron proposes the name "*Micrococcus tetragenus septicus*" for the Koch-Gaffky micrococcus to distinguish it from other similar tetragenous cocci. The latter are nonpathogenic.

Micrococcus lanceolatus.—Synonyms: *Diplococcus pneumoniae,* Pneumococcus of Fraenkel and Weichselbaum, *Diplococcus lanceolatus,* Micrococcus of sputum septicaemia, *Micrococcus pneumoniae cruposae,* etc. Discovered by Sternberg in his saliva in 1880. Demonstrated to be the cause of lobar pneumonia by Fraenkel and by Weichselbaum in 1886. Capsulated, lance-shaped coccus, occurring usually in pairs and short chains, sometimes in long chains. Stains by Gram. Grows best at body temperature; may grow at temperature of 18-22° C. Capable of cultivation on all ordinary alkaline media, but susceptible to slight variations in composition of culture medium. Colonies small, round, gray. Does not liquefy gelatin. Facultative anaerobe. Loses virulence and dies quickly in cultures. May survive in dried sputum or blood for four months. Killed in ten minutes at 52° C. Virulence and other properties extremely variable. Pathogenic for mice and rabbits, in less degree for guinea pigs. Causes localized inflammations and septicaemia.

Present often in the mouth of healthy human beings (page 446). Next to the pyogenic staphylococci and streptococci, it is the most common cause of inflammations in human beings. It is probably the sole specific cause of genuine acute lobar pneumonia, and a frequent cause of bronchopneumonia, otitis media, and meningitis. With or without pneumonia it may cause inflammation in any organ or part of the body. It can produce all kinds of inflammatory exudates—serous, serofibrinous, purulent. It most frequently invades the body from the bronchi or lungs, sometimes from the nose, nasal sinuses, and pharynx, and occasionally from the intestine. The list of diseases which it is capable of producing is a very long one, including inflammations of any of the mucous and serous membranes, abscesses in any part of the body, monarthritis and polyarthritis, osteomyelitis, periostitis, parotitis, thyreoiditis, nephritis, acute ulcerative endocarditis, etc. It may cause septicaemia with single or multiple localizations. It is the most frequent cause of metapneumonic pleurisies, including empyema. Although it may cause the gravest diseases, it is ranked as a relatively benign organism in comparison with *Streptococcus pyogenes,* particularly in pleurisies and suppurations. It may die quickly in inflammatory exudates, as well as in cultures, but it may also persist weeks and months.

Micrococcus pyogenes tenuis.—Found by Rosenbach in pus (1884). Has been described in about twelve cases of suppurative inflammation. It is probably identical with the *Micrococcus lanceolatus* (Neumann).

Micrococcus gonorrhoeae or *gonococcus.*—Discovered by Neisser in gonorrhoeal pus in 1879. First cultivated by Bumm in 1885 on human blood serum. Found constantly in gonorrhoeal pus. The cocci are in pairs, with the adjacent sides flattened against each other, with a clear interspace (biscuit shape). Groups of four with adjacent sides flattened also occur. Particularly characteristic is the inclusion of the cocci within leucocytes, but they occur also free and attached to epithelial cells. Of diagnostic importance is failure to stain by Gram, which distinguishes the gonococci from all the preceding cocci, but not from the so-called pseudogonococci (pp. 456

and 457). Facultative anaerobe. Grows only at body temperature or neighboring temperature. Does not grow on nutrient gelatin or plain agar, or on the latter only with difficulty and occasionally. Grows best on a mixture of human blood serum and nutrient agar (one to two or three parts) (Wertheim). The addition of sterile human urine improves the serum agar mixture (Steinschneider). Surface colonies pale, grayish, translucent, finely granular, with finely notched borders. Forms a membrane in bouillon and blood serum mixed, leaving the fluid clear. Cultures on serum agar when prevented from drying may live forty-five days. Inoculation of pure cultures into healthy human urethrae produces typical gonorrhoea. The gonococcus is a strict human parasite. It dies quickly in dried pus outside of the body. Virulence soon disappears, as a rule, in artificial cultures.

The gonococcus is in general nonpathogenic for animals, but when inoculated with bits of agar into the eye or into the peritoneal cavity of mice and guinea pigs it may cause suppurative inflammation. In human beings its growth is usually superficial, and by preference on mucous membranes covered by cylindrical or transitional epithelium, but it may grow down deeply into connective tissue and between muscle-fibres, and may attack mucous membranes covered by flat epithelium. It is the cause of most cases of salpingitis, pyosalpinx, and the accompanying peritonitis. It may produce genuine abscesses (ovarian, peri-urethral). It may be conveyed by the blood current and cause inflammations in distant parts, most commonly arthritis, also endocarditis, pericarditis, pleurisy, and myocarditis. Every condition of rigid proof of the causation of arthritis by the gonococcus (exclusive presence, isolation in pure culture, and experimental production of gonorrhoea by inoculation) has been fulfilled. It is the usual cause of gonorrhoeal rheumatism. All of these conditions, complicating gonorrhoea, may, however, be produced by other invading microorganisms or be mixed infections with the gonococcus.

Bacillus pneumoniae of Friedländer (*Pneumo-bacillus* or *Pneumococcus* of Friedländer).—Isolated by Friedländer in 1883 from croupous pneumonia. Short plump rods with rounded ends provided with distinct capsules in the animal body. Does not stain by Gram. Facultative anaerobe. Grows at room temperature. Forms prominent, opaque-white, nonliquefying colonies on gelatin. Grows on all media. Ferments glucose and lactose and produces gas bubbles on potato. Pathogenic for mice, less so far guinea pigs, and still less so for rabbits, which, however, may become infected by intraperitoneal inoculation. Found occasionally in the healthy human mouth and nose, also outside of the body. It is found most frequently in inflammations of the mouth, nose, and middle ear. It may cause bronchopneumonia, and has been observed in a very few cases of empyema and of meningitis secondary to middle ear disease and to injury. It is a rare organism in this country.

Bacillus of rhinoscleroma resembles closely the Friedländer bacillus, and according to some, there is no reliable differential character, not even the greater resistance to decolorization by Gram's method, which has been the point chiefly emphasized. There is a group of capsulated bacilli, resembling the Friedländer bacillus, some from human beings, others from animals, which have not been satisfactorily differentiated from each other (see page 451).

Bacillus pyocyaneus.—First cultivated by Gessard from blue pus (1882). A slender, motile, liquefying bacillus, decolorized by Gram, growing rapidly, even at ordinary temperatures, in all culture media. In the presence of oxygen forms bluish, fluorescent green and a whole scale of pigments. Interesting modifications of character, especially as to color production, can be produced artificially, and are observed under natural conditions, constituting, according to some writers, distinct varieties of the bacillus. Is widely distributed, occurring often on the human skin, in the faeces, and outside of the body. In wounds stains the dressings bluish green, and produces a somewhat characteristic offensive odor. Increases suppuration of wounds, usually with little constitutional disturbance. Pathogenic for animals. Is found not infrequently in perforative peritonitis and appendicitis, sometimes in phlegmons, otitis media, bronchopneumonia, and inflammations of serous membranes, associated usually with other bacteria. It was found by Ernst in tuberculous pericarditis. Often found in diarrhoeal and dysenteric discharges. May cause general infections in human beings. With or without general infection it may cause haemorrhagic and necrotic enteritis, a form of pyocyaneus infection in human beings which we have repeatedly observed at autopsy. Instances of invasion of the body from wounds by *Bacillus pyocyaneus* have not been observed.

Bacillus pyogenes foetidus.—First cultivated by Passet from stinking pus of a perirectal abscess (1885). Short bacilli with rounded ends. Cultures have a foul odor; in other respects they do not appear to differ from those of *Bacillus coli communis.* This bacillus probably belongs to the group of colon bacilli. It has been found in pure culture in closed abscesses, but more frequently is associated with other bacteria.

Bacillus coli communis.—Isolated by Escherich from faeces of infants (1886). There is a group of bacilli, called the colon group, presenting similar characters, but with much variation in their cultural and other properties. Short rods with rounded ends, also longer forms. Either motile or nonmotile. Do not stain by Gram in cultures or in the tissues, but do in normal stools. Grow at low as well as high temperatures on all media. Facultative anaerobes. Form large, spreading, grayish white, non-liquefying colonies with notched borders on gelatin and agar, sometimes circumscribed, round, white colonies. On potato, brownish, yellowish, white, or even scarcely visible growth. Coagulate milk, ferment glucose, lactose, and maltose. Constant inhabitants of the intestine, also widely distributed in external nature. The colon bacillus is a frequent invader of the internal organs in all sorts of diseases, especially when there are intestinal lesions. It manifests no evident pathogenic action in most of these cases, and is then without clinical significance. It occurs frequently associated with other bacteria in infected wounds and other inflammations of exposed surfaces. Here also it does not usually appear to cause serious disturbance. The fact that the colon bacillus is so common and widely distributed, and found so often as a harmless invader, should lead to much caution in interpreting the significance of its presence when it occurs in definite lesions. There is no doubt, however, that it may be pathogenic for man. It plays an important rôle in inflammations of the urinary tract and biliary passages; also, but usually with less independence, in peritonitis and appendicitis. The list of diseases in which it may be found is a very long one, and includes inflammations in all organs and parts of the body. Attention has already been called to its pathogenic properties for man (pages 465, 466). In general these properties are of a mild character. One of its leading rôles is to invade territory already occupied by other bacteria or previously damaged. It may be concerned in the production of gall-stones, in the interior of which it has been found by the writer with great frequency. Its virulence as tested upon animals is invariably, but is generally manifest only after inoculation of large doses, which kill by intoxication rather than infection.

Bacillus lactis aerogenes is described by Escherich as shorter and plumper than the colon bacillus. Forms more circumscribed, elevated, white colonies, and coagulates milk and produces gas on potato more quickly and energetically than the latter. Predominates in the upper part of the small intestine. Of late most writers include this bacillus in the colon group, with which it corresponds in its general pathogenic characters. It is sometimes described as an opaque variety of the colon bacillus.

Bacillus typhi abdominalis.—Mention is made of this bacillus, which is chiefly of medical interest, not to describe its characters, but to call attention to its capacity in rare instances to produce genuine suppurative inflammations in man, and especially to cause periostitis and osteomyelitis as a sequel of typhoid fever. Most suppurations accompanying or following typhoid fever are due to the pyogenic cocci or are mixed infections, but the typhoid bacillus may occur alone in abscesses. The most common post-typhoid osseous affection is periostitis with cortical ostitis. The exudate, when caused by the typhoid bacillus alone, may be dark and thin with much detritus, or syrupy in consistence, or genuine pus. The affection is oftener in the ribs than elsewhere, and may be obstinate toward treatment. It may develop several months, and it has been claimed even years, after typhoid fever. Some observations indicate that the typhoid bacillus may cause meningitis and inflammations of serous membranes.

Bacillus proteus.—In 1885, Hauser isolated from putrefying substances the rapidly-liquefying *Bacillus proteus vulgaris,* the slowly-liquefying *Bacillus proteus mirabilis,* and the non-liquefying *Bacillus proteus zenkeri,* which he originally supposed to be three distinct species, but which he now regards on satisfactory evidence as three varieties of the same species, called *Bacillus proteus.* The main characters of this bacillus are its pleomorphism, and especially moving, wandering, irregular projections and islands from its colonies on gelatin and agar (swarming colonies). It is motile. This is one of the most widely distributed bacteria, and is concerned in the decomposition of animal and vegetable material. It is not uncommon in the intestinal contents. Although repeatedly observed in inflammations of exposed surfaces, in appendicitis, in perforative peritonitis, and even in closed abscesses in association with other bacteria, it has been generally considered to be nonpathogenic for man, but our autopsy material has convinced us that it may be pathogenic. It may be unassociated with other bacteria in abscesses and in peritonitis, and it may cause general infection by invading the blood and internal organs (Flexner). In moderate and large doses it is pathogenic for animals.

Bacillus oedematis maligni.—Long spore-forming bacillus resembling the anthrax bacillus, but narrower and with rounded ends. Forms long threads. Strict anaerobe. Liquefies gelatin with gas production and foul odor. Widely distributed in the soil and in the faeces of animals. Pathogenic for animals. No instance is recorded of infection of a previously healthy person with this bacillus, but Brieger and Ehrlich have reported two cases of malignant oedema following the hypodermic injection of musk in a typhoid patient. The bacilli were accidentally introduced by the syringe.

Bacillus aerogenes capsulatus; Bacillus phlegmones emphysematosae.—An anaerobic bacillus first described by Welch and Nuttall in 1892 as the cause of rapid formation of gas in the blood vessels and organs after death. Capsulated thick bacillus, averaging 3 to 6 μ in length. Readily stained by aniline dyes, including Gram's stain. Grows slowly at room temperature, best at body temperature. Produces gas abundantly in all media containing fermentable substances. Does not form spores. When injected subcutaneously or intravenously into animals which are killed shortly afterward; it develops rapidly, with abundant formation of gas throughout the body. The gas is odorless and burns with a blue flame. There is at first no putrefactive decomposition. In the first case reported by Welch and Nuttall the autopsy was made eight hours after death in cool weather, and gas was abundant in the blood vessels and liver. This bacillus is doubtless the cause of the gas formation in many cases reported as death from the entrance of air into the veins, especially from the uterus after abortion, as was proven to be true in a case reported by Graham, Steward, and Baldwin, and in two cases reported by P. Ernst. This bacillus was found in a case of emphysematous gangrene of the hand reported by Mann. It is probably identical with the bacillus found by E. Fraenkel in four cases of emphysematous phlegmon reported in 1893, and called by him *Bacillus phlegmones emphysematosae.* It has been found by the writer in three cases of perforative peritonitis. It may cause emphysema of the subperitoneal tissues. It is probably a rather widely distributed bacillus.

Bacillus diphtheriae.—Observed by Klebs (1883), first cultivated and accurately described by Löffler (1884). Straight or slightly curved rods, averaging 1.2 to 2.5 μ in length, characterized especially by irregularities in shape and staining. Particularly characteristic are swollen ends and deeply staining chromatin granules in the bacilli. Stains by Gram. Not motile. Does not form spores. Facultative anaerobe. Optimum temperature for growth 33-37° C. Grows only slowly and slightly, if at all, below 20° C. Grows on all alkaline culture media, invisibly on potato, best on Löffler's blood serum bouillon mixture. Does not liquefy gelatin. Forms on agar grayish white, granular colonies with slightly irregular margins. In bouillon grows in the form of small grayish particles, with or without clouding of the medium, often with formation of surface membrane. Particularly important as a diagnostic criterion is the change of the alkaline to acid reaction by forty-eight hours' growth in bouillon containing carbohydrate. Thermal death-point, 58° C. in ten minutes. May survive in some culture media eighteen months, but may die in three or four weeks. Lives longer in the dark than when exposed to light. Resistant to desiccation. May survive for three to five months in dry membranes, but usually dies sooner. Cultures dried on threads survive three to four weeks at room temperature. Both virulent and nonvirulent diphtheria bacilli occur, the latter rarely in diphtheria. Pathogenic for many animals, and especially for guinea pigs. By subcutaneous inoculation there is produced extensive local inflammation and necrosis. The animal dies usually in thirty-six to sixty hours, with necrotic foci in various internal organs and serous transudates in the serous cavities. The bacilli are found only near the seat of inoculation or in very small number in the organs. With less virulent cultures or smaller doses subacute and chronic infections or intoxications ensue. Genuine pseudomembranes, as a rule with little tendency to spread, follow inoculation of mucous membranes superficially injured. The virulent diphtheria bacillus produces by its growth in cultures or in the animal body a powerful poison, called the toxin or toxalbumin of diphtheria, to which the constitutional symptoms, the lesions of internal organs, and the paralysis are due.

The diphtheria bacillus is the cause of all cases of genuine diphtheria. Similar pseudomembranous inflammations of the throat and air passages may be caused by streptococci. The intensity of the affection caused by the diphtheria bacillus varies from a slight inflammation without false membrane to the gravest pseudomembranous inflammations.

The diphtheria bacillus is incapable of attacking the intact skin. It may, however, produce pseudomembranous inflammations on excoriated, ulcerated, and wounded skin. Neisser has reported the case of a child five and a half years old with diphtheria of the throat in whom thick, firmly adherent pseudomembrane covered the skin about the anus over a space 10 cm. long and 4 cm. broad. There was oedema of the scrotum and penis. Löffler bacilli were found in the false membrane and throughout the infiltrated corium. The localization of the diphtheria bacillus in cutaneous surfaces which have been deprived of the epithelial covering by excoriation, eczema, ulceration, herpes, wounds, has been observed many times in persons affected with diphtheria of the throat, although it is not common. This localization may be attended by a pseudomembrane, or by simple superficial necrosis, or by ordinary suppuration or inflammation in no way suggesting diphtheritis. Park found diphtheria bacilli in two cases in wounds of the finger received by physicians in intubating children with diphtheria. They persisted for six weeks in one case. Wright has demonstrated the presence of this bacillus in excoriated or ulcerated surfaces of the skin in 7 cases, in paronychia in 1, in mastoid abscess in 1, in purulent conjunctivitis in 1—all in cases of diphtheria. The diphtheritic process may extend by continuity to a tracheotomy wound. More frequently the wound is unaffected, even in cases where the diphtheria bacillus may be demonstrated upon its surface. Extensive necrotic and septic involvement of tracheotomy wounds is more frequently due to streptococci, with or without association with the diphtheria bacillus, than to the latter microorganism alone. Brunner has reported three cases of infection of wounds with the diphtheria bacillus in association with pyogenic cocci in persons without demonstrable connection with other cases of diphtheria and without any affection of the throat. In only one case was there pseudomembrane. The presence of the diphtheria bacillus was not suspected in any of these cases before the bacteriological examination.

In this connection a few words may be said regarding the general subject of wound diphtheritis, concerning which much confusion exists. Here, as well as in diphtheritis of mucous membrane, it is important to bear in mind that the term "diphtheritis" is a purely anatomical one, and implies nothing as to the causation of the affection. The term "diphtheria," on the other hand, should be restricted to the affection caused by the Löffler bacillus wherever this may be localized. There are three anatomical conditions of a wound to which the epithet "diphtheritic" has been applied—namely, first, the presence of an adherent fibrinous false membrane incorporated with underlying necrosis of the tissues; second, the presence of a fibrinous pseudomembrane loosely attached to the underlying tissues, which may present no distinctive alteration; and, third, more or less extensive necrosis of the tissues of the wound without a distinct false membrane. Only the first condition is properly called diphtheritis; the second may be called simple pseudomembranous or croupous inflammation, and the third is necrotic inflammation.

Wound diphtheria is infection of a wound by the Löffler bacillus. It may or may not be wound diphtheritis in the anatomical sense. Wound diphtheria may manifest itself as a simple inflammation, or inflammation with superficial necrosis, or inflammation with more or less adherent pseudomembrane. Pyogenic cocci are usually associated with the Löffler bacillus in the diphtheria of wounds. Paralysis may follow wound diphtheria. As the same anatomical conditions in a wound may be produced by various causes, a positive diagnosis of wound diphtheria can be made only by bacteriological examination. The conditions as regards varying intensity and character of the infection, association with other bacteria, particularly streptococci, and the necessity of a bacteriological examination to establish the diagnosis, are in no way different in the diphtheria of wounds from those in diphtheria of mucous membranes. As has already been stated, wound diphtheria may occur without demonstrable connection with cases of diphtheria and without affection of the throat in the individual attacked, but such occurrences are rare.

Diphtheritic, necrotic, and croupous inflammations of wounds are caused most frequently by other microorganisms than the Löffler bacillus. Here, as with similar inflammations of mucous membranes, *Streptococcus pyogenes* appears to be an important causative agent, so far as can be judged from the small number of cases hitherto examined bacteriologically. In this comparatively rare class of streptococcus infection of wounds the intensity and extent of the inflammation vary within wide limits.

It is not, or formerly was not, a particularly rare occurrence for coherent false membranes to develop upon granulating surfaces without notable disturbance in the process of repair and without constitutional disturbance. The membrane in these cases can be readily stripped off, after which another membrane is likely to form. It has been proposed to call this relatively inoffensive wound complication "wound croup," in distinction from the more severe wound diphtheritis. In two cases of croupous membranes on granulations Brunner found *Streptococcus pyogenes* in pure culture. In similar cases he and Tavel have found also *Bacillus coli communis* in pure culture. The condition may doubtless be associated with the presence of various microorganisms, and is not, therefore, a specific one. In croupous inflammations of fresh wounds Brunner found *Streptococcus pyogenes* in association with other pyogenic cocci.

How much the development of diphtheritic and necrotic inflammations of wounds is influenced by the general condition of the patient is shown by the greater frequency of its occurrence in persons prostrated with typhoid fever, scarlet fever, septicaemia, pyaemia, and other debilitating causes.

Diphtheritic inflammation of operative wounds involving the mouth and the bladder is more common than a similar affection of wounds in other parts of the body. The Löffler bacillus is not concerned, as a rule, with diphtheritis of these wounds, any more than it is with diphtheritic cystitis or enteritis.

Much interest attaches to the question of the causation of hospital gangrene, that frightful scourge of pre-antiseptic surgery in crowded hospitals, particularly in military hospitals. Hospital gangrene has been designated "wound diphtheritis," but it differs from the necrotic and diphtheritic inflammations of wounds, which are now occasionally observed, in many features, particularly by its phagedenic character and its mortality. It has apparently disappeared, at least from civilized countries, and there has been, no opportunity to make a bacteriological examination by modern methods of any case of this disease. Although some of the older writers identified the cause of hospital gangrene with that of true diphtheria, it seems, with our present knowledge, highly improbable that this is true. Our observations of the effects produced by the diphtheria bacillus with or without association with other bacteria in wounds do not indicate that this organism can have played any essential rôle in the causation of hospital gangrene. Some authorities are of the opinion that hospital gangrene is a specific infection due to a specific microorganism which has disappeared from civilized countries, as the microorganism of the plague has disappeared. It seems to the writer more probable that it was due to pyogenic bacteria which still exist, but which under the special conditions prevailing where hospital gangrene occurred had acquired a degree and kind of virulence with which we are no longer familiar. Thanks to antiseptic surgery, these special conditions are not likely to be repeated in civilized countries.

Bacillus tetani.—Observed by Nicolaier (1884) in wounds in cases of tetanus and demonstrated in garden earth, first obtained in pure culture by Kitasato (1889). Slender, straight bacilli, varying in length from short rods to long threads. Sluggishly motile. Spherical spores, thicker than the rods, develop at one end of the bacilli, giving them a drumstick or pin shape. Stains by Gram. Anaerobic. Grows best at body temperature, slowly at 18-20° C., not at all below 14° C. Spores are formed in cultures at body temperature in thirty hours, in cultures at 20° to 25° C., not until after a week. Grows in nutrient gelatin, which is slowly liquefied with slight gas production; in agar and bouillon when free oxygen is excluded. Growth in gelatin is fuzzy, radiating, like a thistle. Dried spores live months and years. Cultures containing spores dried on silk threads were found alive after several months by Kitasato. Henrijean demonstrated living, virulent tetanus spores on a piece of wood which had been extracted eleven years previously from the wound in a boy with fatal tetanus. The spores are killed in five minutes by moist heat at 100° C. They withstand in the moist condition for an hour at a temperature of 80° C.—a property which was utilized by Kitasato to destroy other bacteria in obtaining pure cultures of the tetanus bacillus. The spores survive and preserve their virulence for ten hours in 5 per cent carbolic acid; they are killed in fifteen hours. They are not killed by putrefactive bacteria.

The tetanus bacillus is more or less pathogenic for nearly all warm-blooded and some cold-blooded animals. Infection takes place only through a wound. The period of incubation varies from a few hours to several days, according to the susceptibility of the animal and the dose. Experimental tetanus corresponds in all essential particulars to human tetanus. Tetanic spasm appears first in the muscles nearest the seat of inoculation.

Tetanus is a toxic infection. The bacilli multiply only in the immediate neighborhood of the wound, and do not invade the blood and organs. Inoculation with pure cultures is a pure intoxication as a rule, with but little multiplication of the bacilli, so that at the autopsy it may be difficult to find any bacilli. When impure cultures and foreign bodies are introduced into the wound the bacilli are more readily demonstrable at autopsy. There are no demonstrable lesions of internal organs in experimental tetanus.

All of the symptoms of tetanus can be produced by the germ-free filtrate of tetanus cultures. It is the only infectious disease known every feature of which can be produced experimentally by injection of the poison without the microorganisms. In the case of diphtheria all of the symptoms and lesions can be produced by the poison except the local false membrane. This requires the presence of the bacilli.

Brieger found in impure tetanus cultures four crystallizable alkaloidal substances belonging to the class of ptomaïnes. Three of these he called tetanin, tetanotoxin, and spasmotoxin. He found also an unnamed base. As these ptomaïnes produced spasms by injection into animals, this result was at first though to indicate that the poison of tetanus had been isolated in a state of chemical purity. Subsequent investigations have shown that these ptomaïnes do not reproduce all of the characteristic symptoms of tetanus. They are not the real tetanus poison, and no particular interest any longer attaches to them.

The real poison of tetanus belongs to a different class of substances—namely, the so-called toxic proteids or toxalbumins, substances about which we know very little chemically, but a great deal physiologically. We do not even know positively that the tetanus toxin is a proteid, but it is generally assumed to be. To this same class of substances belong the toxin of diphtheria, the venom of snakes, and certain poisonous substances produced by vegetable cells, as ricin, abrin, robin.

The specific tetanus toxin has been demonstrated both in cultures of the tetanus bacillus and in the bodies of animals. It has been found in the blood both during life and after death. It does not appear to be eliminated in appreciable amount by the urine unless very large doses are given. According to Kitasato, the poison is destroyed in the presence of water in five minutes at 65° C., in one and a half hours at 55° C.; it stands drying at ordinary temperatures; is not injured by dilution with water or bouillon; is sensitive to acids and alkalies. It is destroyed by the acid gastric juice. It is injured by exposure to light. So sensitive is the tetanus poison to chemical reagents that Kitasato was unable to find any means of obtaining the poison in a condition approaching purity, and he expressed ignorance as to its real nature. Brieger and Cohn have, however been more successful, and have isolated a substance possessing the properties of the tetanus poison in a condition approaching purity. As this substance was found to be without most of the proteid reactions, they consider that it is not an albumin in the ordinary sense of that term.

The tetanus toxin is of appalling potency. Kitasato obtained liquid cultures of such virulence that 0.00001 c. c. of the germ-free filtrate, corresponding to 0.00023 mgm. of the dried filtrate, sufficed to kill a mouse with tetanus. Of course only a part of the dried substance is the real poison. Of the purified substance obtained by Brieger and Cohn, who had at their disposal cultures of less primary virulence than those of Kitasato, 0.000,000,05 gm. killed a mouse of 15 gm. weight. As the fatal dose of the tetanus poison increases with much regularity in proportion to the weight of the animal, this would represent 0.23 mgm. as the fatal dose for a man weighing 70 kilo. When it is considered that the minimal fatal dose of atropine for an adult is 130 mgm. and of strychnine is 30-100 mgm., some conception of the terrible energy of this bacterial weapon can be obtained. The substance separated by Brieger and Cohn was not in a state of chemical purity. The tetanus poison, like the diphtheria poison, does not cause symptoms immediately after its introduction. With small doses it may be days before recognizable symptoms appear. This behavior is unlike that which we are accustomed to attribute to chemical poisons, and raises the question whether the poison may not be reproduced in the body, or whether the substance injected is itself the poison, and may not be in the nature of an enzyme which leads to the production of the real poison within the body. These questions cannot at present be answered.

An interesting example of the effect of the pure tetanus toxin upon man is reported by Nicolas. In working with the filtrate of a tetanus culture he accidentally stuck the point of the needle of a hypodermic syringe containing some of the fluid into his left hand. Only the moisture adhering to the needle was introduced into the puncture. After three and a half days the first symptoms of tetanus were manifested by contracture of the left thumb. There followed in succession contraction of the hand, the arm, then trismus, opisthotonos, general contractures, and convulsions. The treatment was by large doses of chloral. After three weeks improvement began, and recovery was complete after forty-one days. In this unique case the tetanus toxin was introduced in minimal amount without the bacilli. Nicolas, in view of the fact that no symptoms were manifested until the third day, adopts the view that the culture fluid does not contain the real poison, but produces it by a kind of fermentation after introduction into the body.

The distribution of the tetanus bacillus and the factors favoring infection with tetanus have already been described (pp. 441, 455, 468, and 484).

Susceptible animals may be rendered immune from tetanus by the injection of the tetanus poison, at first weakened by chemical agents or heat, and then administered in full strength in constantly increasing doses. This is antitoxic immunity, already described, which has nothing to do with natural immunity. The hen is naturally immune from tetanus, but not by virtue of any antitoxic power of the blood. The blood serum and other fluids of animals rendered artificially immune from tetanus are capable, by virtue of their antitoxin, or rendering susceptible animals resistant to tetanus (passive immunity), or even of preventing the development of the disease or of curing the disease after reception of the virus. Behring in 1892 reported that in the course of two years he had by successive injection of increasing doses of the tetanus toxin rendered a horse so highly immune that the immunizing value of its blood serum was 1 to 10,000,000, by which is meant that $\frac{1}{10000000}$th cm. of serum will protect 1 gm. weight of mouse from the effects of the subsequent injection of the smallest fatal dose of the tetanus poison, or 1 c. c. of serum suffices to immunize 500,000 mice, weighing each 20 gm., or 200 sheep, weighing each 50 kilo. When, however, the attempt is made to prevent the development of the disease immediately after the reception of the virus, a larger quantity of the serum is required to afford protection than a few hours previously, and as time elapses the amount of serum required rapidly increases, until, as soon as the very first symptoms of the minimal fatal dose of tetanus poison appear in the mouse, one thousand times the quantity of serum necessary for simple preventive immunization must be injected. After twenty-four hours the necessary quantity of serum possessing an immunizing value of 1 to 1,000,000 is too great to be introduced into the animal. To produce the same curative effects in large animals, as in small it is necessary to inject proportionately larger amounts, the dose being in approximately direct ratio to the respective weights of the animals. These considerations manifestly suggest serious difficulties in the application of the antitoxic treatment of tetanus to human beings. But there is another difficulty of probably greater force—namely, that we have no indication that tetanus will result from a wound in man until characteristic symptoms have appeared. By that time tetanus poison has accumulated in considerable amount in the system. Kitasato was unable to prevent the development of tetanus in mice by the complete excision and thorough cauterization of the wound one hour after inoculation with tetanus bacilli. It is not positively proven that curative antitoxic serum neutralizes poison which has already been received into the system before injection of the serum, although it is capable of neutralizing poison formed after its administration. We stand in a much less favorable position for the successful treatment of human tetanus by antitoxin than is the case with diphtheria, where the conditions are more favorable both as regards progressive increase of dosage in proportion to the weight, and especially as regards the possibility of beginning treatment before the absorption of large quantities of the poison. These are the scientific considerations relating to the treatment of tetanus by antitoxin. Its practical application and the results of treatment in human beings belong to the article on "Tetanus" in: Syst. Surg. (Dennis), Phila., 1895.

Bacillus tuberculosis.—Discovered and first cultivated by Koch (1882), independently and at about the same time demonstrated microscopically in tubercles by Baumgarten. Slender, straight, or slightly curved or bent, nonmotile rods, 1.5 to 4 μ in length, which averages five to six times the breadth. In unstained specimens glistening dots, in stained specimens clear spots, are often seen in the rods, which thereby present a beaded appearance. These are interpreted by Koch and others as spores, but this has not been proven. No greater resistance to heat and other injurious agencies has been demonstrated for bacilli containing these suspected spores than for bacilli without them. Tubercle bacilli take up staining dyes with difficulty, but when once thoroughly stained by intense aniline dyes they retain the color after it has been extracted by acids, alcohol, and other decolorizers from all other known bacteria except the leprosy bacillus and some examples of smegma bacilli. (See p. 442.) It is by this peculiar staining reaction that tubercle bacilli are differentiated from other bacteria. The tubercle bacillus grows best in the presence of free oxygen, but is capable of growth without free oxygen. Optimum temperature for growth, 37° to 39° C. Does not grow below 29° C. Was first cultivated by Koch on solidified cow's blood serum; has since been cultivated on potato, and on nutrient agar and bouillon to which 5 to 6 per cent glycerin has been added. On blood serum the growth first becomes visible after ten to fourteen days. It forms dry, lustreless, compact white scales, loosely attached to the surface. By coalescence of the colonies extensive membranous growths are formed. Grows in the form of a membrane on the surface of fluid media, without clouding the fluid. The tubercle bacillus is the most resistant to heat of all pathogenic bacteria not positively proven to form spores. The statements of different experimenters as to its thermal death-point are so widely divergent that bacilli from different sources, or at least under different conditions, would seem to vary in this respect; but it is more probable that the discordant results are due to failure to ensure exposure of the bacilli to the actual temperature of the surrounding fluid. The physical conditions as regards distribution of the temperature in heated animal fluids, culture media, etc., especially when solid masses are present, are not of so simple a nature as many of the experimenters upon the subject of the thermal death-point of bacteria seem to suppose. According to the careful experiments of Forster, tubercle bacilli are killed, or at least are rendered incapable of infecting guinea pigs, by ten minutes' exposure to a temperature of 70° C. and by one hour's exposure to 60° C. They were not killed in forty-five minutes by heating to 60° C. They are surely killed in a few minutes by the boiling temperature. They retain their vitality in dried sputum for nine or ten months, but at the last with some loss of virulence. They resist putrefaction for a considerable time. They are killed in sputum by 3 per cent carbolic acid in twenty hours; they are killed in a few minutes by 3 to 5 per cent carbolic acid when the acid can come into direct contact with the bacilli. They resist the action of the gastric juice. When exposed in thin layer to the direct action of the sun's rays they are killed in a few minutes—much more slowly when in a thick layer.

The tubercle bacillus is a strict parasite, incapable under ordinary conditions of multiplication in the outer world, but capable of prolonged survival outside of the body. It is the sole cause of tuberculosis. The pathogenic manifestations of the tubercle bacillus have been described by Dr. Councilman (Syst. Surg. (Dennis), Phila., 1895, Vol. I, p. 239), and will be further considered in the articles treating of the various forms of surgical tuberculosis.

The bacillus of avian tuberculosis is a different species, or at least a different variety, from that of human tuberculosis.

Bacillus leprae.—Discovered by Hansen (1879) in leprous tubercles. Morphologically, the leprosy bacillus resembles very closly the tubercle bacillus, from which it probably cannot be distinguished in size and shape. It presents clear, unstained dots like those observed in tubercle bacilli. It resembles the tubercle bacillus also in its staining reactions, the only important difference being that it is more readily stained by aniline dyes than the tubercle bacillus. It stains well by Gram's and Weigert's fibrin stain. It is nonmotile. Although many observers claim to have cultivated the leprosy bacillus, none of these claims have been established, at least not to the satisfaction of most bacteriologists. Most experimenters have had only negative results from the inoculation of leprous material into animals. The positive results reported by Damsch, Vossius and Melcher, and Ortman are open to criticism in their interpretation. The apparently successful inoculation of a condemned criminal in the Sandwich Islands by Arning has also been criticised as not conclusive. The constant and exclusive presence of the bacillus leprae in leprosy cannot be reasonably interpreted otherwise than that the bacillus is the cause of the disease. The characteristic bacilli are present in enormous number in the leprous nodules, being chiefly enclosed within cells. They have also been found in the lesions of the disease in all parts of the body. They are very rarely in the blood.

Bacillus mallei.—Discovered by Löffler and Schütz (1882) in the lesions of glanders. Somewhat shorter and thicker than the tubercle bacillus. Presents often clear, unstained spaces in the rods. It probably does not form spores. Decolorized by Gram. Stains with the usual aniline dyes, but is so easily decolorized that its demonstration in sections is somewhat difficult. Like the typhoid bacillus, it occurs in the tissues especially in clumps. Nonmotile. Facultative anaerobe. Grows best at body temperature, but is capable of growth at room temperature. Grows on all culture media, but, as has been shown by Theobald Smith, far better on acid (nonneutralized) than on alkaline media. Particularly characteristic is the growth on potato, on which the glanders bacillus forms at first a translucent, amber yellow layer, later a reddish brown layer with discoloration of the potato. Growth on agar whitish, moist; if the medium be acid, thick and abundant. The virulence diminishes in successive generations in artificial cultures. The glanders bacillus may survive in the dried condition for three and a half months, but usually dies within two or three weeks. There are observations which indicate that it may survive for at least a year and a half in unoccupied, infected stables. By inoculation of horses with pure cultures glanders is produced. The bacillus is pathogenic for several species of animals. The guinea pig and the field mouse are particularly susceptible. White mice are resistant. The field mouse dies from experimental inoculation usually in three or four days with acute infection and minute tubercle-like nodules in the spleen and liver. The lesions in the guinea pig are most characteristic, consisting in a caseous ulceration at the seat of subcutaneous inoculation, which, however, is often absent; swelling and necrotic suppuration of the testicles; swelling and ulceration of the joints; and nodules in the spleen and liver, sometimes elsewhere. The best method of diagnosing a suspected case of glanders is to inoculate the material into the peritoneal cavity of a male guinea pig. In four or five days, at the most in eight or ten days, the characteristic swelling and inflammation of the testicles can be detected. The bacilli are in the lesions, but scanty or absent in the blood. Mallein is a product derived from cultures of the glanders bacillus, and is analogous in its properties and uses to tuberculin derived from the tubercle bacillus. It is used for the diagnosis of glanders in animals, as tuberculin is used for the diagnosis of tuberculosis.

Bacillus anthracis.—Discovered in the blood of animals affected with anthrax by Pollender (1849); also observed by Davaine (1850), Rayer (1851), and Brauell (1857). The studies upon the anthrax bacillus laid the foundation-stone of modern bacteriology. The bacillus is 1 to 1.5 μ broad and 3 to 10 μ long. It grows out into long threads made up of bacilli, the adjacent ends of which are sharply cut and slightly concave. Not motile. Stains readily with aniline dyes, including Gram's stain. Forms spores in the presence of oxygen, but never within the animal body. Grows at room temperature, best at body temperature; does not grow below 12° C. Grows on all culture media. Liquefies gelatin with moderate rapidity. Colonies on gelatin are whitish, often with fuzzy, irregular, hair-like projections. Similar bristle-like projections often characterize the growth in the line of puncture in gelatin and agar. Desiccated spores may survive for years. Anthrax spores are usually killed in four minutes by boiling temperature, but they vary in their resistance, and may withstand boiling temperature for twelve minutes (von Esmarch). Some anthrax spores are killed by 5 per cent carbolic acid in two days; others survive for forty days. Various modifications of character of the anthrax bacillus can be produced by cultivation at high temperatures (42-43° C.) or by the addition of dilute antiseptics, the most important modifications being the production of an asporogenic variety of the bacillus and the loss of virulence. Any degree of attenuation of virulence can be produced down to complete loss. These attenuated cultures serve as vaccines to render animals insusceptible to anthrax. The anthrax bacillus is a facultative parasite. It is in the highest degree virulent for mice and guinea pigs, somewhat less so for rabbits. Sheep and cattle are the animals which suffer the most from the natural disease. Swine, dogs, and most birds are immune. Rats and pigeons are resistant, but not wholly insusceptible, to anthrax. Man does not rank among those highly susceptible to the disease. Infection may occur from the skin, intestine, or lungs. In small susceptible animals experimental anthrax is a septicaemia with abundant bacilli in the blood. In man anthrax usually occurs with localized inflammations at the seat of invasion (malignant pustule, intestinal anthrax, woolsorter's disease). In some cases the disease remains localized; in others general infection and multiple localization occur.

Actinomyces, or ray fungus.—First recognized as a living organism by Bollinger in 1877 in actinomycosis or lump-jaw of cattle; observed in man in 1845 by B. von Langenbeck, whose discovery, however, was not published until 1878, when it was mentioned by J. Israel, to whom belongs the credit of first recognizing the ray fungus as a distinct microorganism pathogenic for man.

There are various species or varieties of the genus actinomyces, but we are able with our present methods to differentiate them from each other only imperfectly. *Actinomyces hominis,* which is probably identical with *Actinomyces bovis,* is the only species which will be considered here. Authorities are not agreed as to the botanical position of this organism, but most writers now regard it as belonging to the group of the more highly organized pleomorphic bacteria, and allied to cladothrix or streptothrix. Some, however, class actinomyces with the fungi.

Actinomyces is the cause of a chronic inflammatory affection, presenting sometimes the characters of an abscess, at other times more those of a tumor, with suppurating tracts and cavities. The presence of the parasite can usually be detected with the naked eye by finding in the pus small yellowish granules. These granules vary in size, shape, and color. They are usually described as about the size of a pin's head. They may be much smaller, even microscopic in size, and exceptionally they may be massed together in clumps the size of a pea. To the naked eye they generally appear round, but microscopically their contours are seen to be irregular. They may be grayish and translucent like sago particles, or opaque gray or white, or yellow, greenish yellow, brownish, even black. Bostroem has demonstrated that these various tints depend partly upon the stage of development of the organism, the younger forms without club-like terminations being pale and transparent. The black granules have been observed only in intestinal actinomycosis. The consistence of the granules or colonies in their early stage of development is soft and gelatinous; later it becomes firm and compact, and it may become hard and gritty from deposit of lime salts in the old, degenerated colonies. The young, translucent bodies are most abundant in foci of rapid softening and liquefaction of the tissues. The number of the bodies present in the pus varies in different cases. They may be so few as to require long search to find them; usually they are numerous, and sometimes there are myriads of them.

These characteristic granules, visible to the naked eye, are colonies of the actinomyces or clumps of colonies. The youngest colonies are not visible to the naked eye. These consist of a central interlacing mass of branching threads with radiating projections of threads. The most common characteristic appearances are found in older colonies (called by the Germans "Drusen"). Here we find the centre of each colony composed of extremely fine interlacing threads and small round bodies resembling cocci, and the periphery made up of bulbous, club-like terminations of the threads. It is particularly this outer ring of radiating, club-shaped projections which gives to these bodies their most diagnostic feature. It requires careful study and differential staining to make out the real structure of the actinomyces bodies. Ordinarily, the central part of the colony appears simply as a confused granular and finely filamentous mass. It has, however, been shown to consist of extremely delicate rods and threads, which branch dichotomously, and which, arranged somewhat loosely in the centre, become more densely interwoven and larger toward the periphery, and there pass in a radiating manner one into each of the swollen bulbs. This bulbous swelling appears to be due to an accumulation of a clear hyaline material in the sheaths of the fibers, and to represent only an involution or degenerative condition, not forming an essential part of the living structure of the parasite. The hyaline bulbs may present an appearance of concentric striation, and each one may break up into finger-like projections or have somewhat the appearance of a pine cone. They may become calcified. These are all degenerative changes. Both within and outside of the threads are seen round bodies like cocci, which are considered by Bostroem to be spores, but whose nature is undetermined. They may be very abundant, so that the central part of the colonies consists almost wholly of these round granules with very few threads. Cases of actinomycosis occur exceptionally in which there are no bulbous endings to the threads composing the colony. In addition to the colonies, numerous bacilli are often found scattered throughout the inflammatory nodules of actinomycosis. Pyogenic cocci are frequent secondary invaders in actinomycotic growths. Gram's and Weigert's fibrin stain and haematoxylin and eosin can be used to advantage in the microscopical study of actinomyces.

Many investigators claim to have cultivated the ray fungus. There is so much discrepancy in their descriptions of the characters and conditions of these cultures that we cannot recognize all of them as genuine cultures of actinomyces. Probably the most confidence is to be given to the cultures obtained by J. Israel and M. Wolff. They found that actinomyces grows best anaërobically and at the temperature of 35°-37° C. They used chiefly as their culture media nutrient agar and hen's and pigeon's eggs. The growth is slow and in the form of dry, small, projecting, irregular colonies, which are at first gray and translucent, and later opaque. On agar actinomyces grows chiefly in the form or rods—in eggs, in the form of interlacing, branching threads. Bulbous terminations of the threads are not observed in the cultures.

The majority of experimenters have obtained only negative results in attempting to produce actinomycosis experimentally in animals by inoculation of material containing the specific microorganism. Bostroem in his elaborate article on actinomycosis considers all of the positive results reported up to the date of its publication (1890) as inconclusive. Since that date, however, several observers have reported more or less success in producing the disease experimentally; and here, again, the results obtained by Israel and Wolff are the most noteworthy. They succeeded by introduction of their pure cultures of actinomyces into the peritoneal cavity of rabbits and guinea pigs in producing small nodules in the peritoneum and spleen. These nodules contained typical actinomyces colonies with bulbous rays. Further investigation is needed regarding the experimental inoculation of actinomyces.

Infection both of human beings and of cattle with actinomycosis has been often traced to the penetration of vegetable grains (wheat, barley, oats) or bits of vegetable fibre into the tissues. The portal of entry is frequently the mouth, particularly the neighborhood of the teeth, but it may be through a wound of the skin or through the mucous membranes of the throat, respiratory tract, oesophagus, intestine, or middle ear. It is not always possible to find the point of entrance, as this may heal completely.

Various clinical types of actinomycosis are recognized according to the seat of affection, such as actinomycoses of the jaws and elsewhere about the head and neck, of the lungs, the pleura, the vertebrae, the intestine and other abdominal organs, the brain, the middle ear, the skin. There is usually a single localization, but there may be multiple foci, presenting sometimes anatomical and clinical characters of pyaemia.

The histological changes are characterized by necrosis and liquefaction of tissue with emigration of white blood corpuscles as the immediate effect of the parasite, and a secondary reactive inflammation in the form of vascular granulation tissue. By some the changes are considered to be more closely allied to those of infectious granulomata than of ordinary inflammation. In some cases the process is rapid and destructive, with little new formation of tissue, and with the production of large abscesses; in others the affection may be very chronic, of limited extent, and encapsulated by new growth of tissue. When bone is involved there may be formed osteophytes and sclerosing ostitis, or there may be simply destruction without new formation of bone. Metastases do not appear to occur in the lymphatic glands communicating by the lymph-current with a primary focus of actinomycosis.

Madura disease, or mycetoma, both in the melanoid and the ochroid varieties, has been shown by Kanthack to be due to a parasite very closely allied to the ordinary *Actinomyces hominis*. Kanthack considers that the parasite is simply a variety of the ordinary actinomyces.

A GAS-PRODUCING BACILLUS (*BACILLUS AEROGENES CAPSULATUS,* NOV. SPEC.) CAPABLE OF RAPID DEVELOPMENT IN THE BODY AFTER DEATH[1]

(*From the Pathological Laboratory of The Johns Hopkins University and Hospital*)

There are to be found in medical literature many cases in which the blood vessels after death contained air or gas which did not seem attributable to post mortem decomposition. Various explanations of this occurrence have been offered. The observation to be here reported is calculated to shed light upon some of these mysterious cases. The microorganism to be described is in itself also of much interest.

The following is an abstract of the clinical history and post mortem examination of the case:

J. M., male, light mulatto, aged 38, bricklayer. Admitted to the hospital, service of Dr. Osler, Oct. 24, 1890; discharged July 15, 1891; readmitted Oct. 24, 1891; died Oct. 25, 1891. Patient has used alcohol to excess and gives a history of syphilis. For two years has suffered from pain in the chest, dyspnoea, and paroxysmal cough. Spat blood three years ago. Six weeks before admission first noticed a pulsating lump on the right side of the chest. Upon admission there is observed a large, hemispherical, pulsating tumor in the right infraclavicular and mammary regions, reaching the border of the sternum, and most prominent in the second intercostal space. The skin over the tumor is intact and brownish in color. The manubrium sterni near the second costal cartilage is eroded. Percussion note over tumor is dull. Apex beat of heart in 5th interspace. No cardiac murmurs. Cardiac dullness not increased. No tracheal tugging. Arteries stiff, tension increased, pulse 84. No difference between radials. Pulmonary râles heard in scapular regions. While in the hospital the tumor gradually increased in size, extending from the sternoclavicular articulation to the nipple, with complete erosion of sternum over about one-half its width. Thoracic and epigastric veins distended. Urine free from albumen and casts. Patient left the hospital in July, 1891. When readmitted on October 24, 1891, he reports that his condition remained about the same until a month ago, when

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[1] William H. Welch and George H. F. Nuttall, M. D., Ph. D. (Associate in Bacteriology and Hygiene, The Johns Hopkins University).

A brief report of this case under the title, Aneurism with Demonstration of Bacilli Causing Air in the Tissues, and description of the bacillus were presented by William H. Welch before The Johns Hopkins Hospital Medical Society on November 2, 1891.

Johns Hopkins Hosp. Bull., Balt., 1892, III, 81-91.

a small ulcerated opening formed over the tumor, through which he lost about a pint of blood. A week later a larger rupture occurred on coughing, and he says that he lost about a gallon of blood, some coming through the mouth. A third haemorrhage occurred the night before readmission, when the blood spurted through both external openings, to the extent, he says, of about a gallon. Upon readmission the patient's condition did not appear to be immediately alarming. His mind was clear, appetite good, and there was no dyspnoea. The urine contained a small quantity of albumen with hyaline and granular casts. The patient died suddenly, without any further loss of blood, at 6 o'clock A. M., October 25. In view of what was found at the autopsy, it may here be stated that no emphysematous swelling of the neck and no escape of gas from the ruptured openings of the aneurism were observed during the short time the patient lived after his reentrance to the hospital, or immediately after his death, but no special attention was directed to these points.

The *post mortem examination* was held eight hours after death, the weather being cool. The body was still warm and presented no discoloration of the skin of the abdomen or other signs of decomposition.

Rigor mortis present. Considerable emaciation. Puffiness of lower eyelids. Diffuse livid redness of face. Lower portion of neck up to the upper level of thyroid cartilage diffusely and symmetrically swollen, with emphysematous crackling, extending on the right side posteriorly to the vertebrae, on the left most evident anteriorly and laterally. Emphysematous crackling can be felt also in both axillae, on the inner side of the arms, over the pectoral muscles, over the buttocks, in the groins and the inner side of the thighs. The subcutaneous veins over the abdomen and thorax, especially on the right side, are distended, tortuous, and contain gas bubbles. Emphysematous crackling can be felt along the track of the superficial veins and over most of the body and extremities; it is limited to them.

Anteriorly on the right side of thorax is a tumor, with fluctuating feel, projecting about 4 cm. above the level of the skin, and extending from the first rib downward a distance of 12 cm., and laterally from the right margin of the sternum 11 cm., the most prominent part being 4 cm. from the sternal border. Near the most prominent part of the surface of the tumor are two circular openings through the skin, 1 cm. and 0.5 cm. in diameter, from which thin bloody fluid, mixed with gas bubbles, escapes.

Before making the usual incisions into the abdomen and thorax, a number of veins and arteries, including the jugular, femoral and brachial veins, superficial veins of abdominal wall, femoral and temporal arteries, are carefully exposed by small suitable incisions through the skin covering them, and the presence of gas bubbles is determined in all of them without opening them. The presence of gas in the subcutaneous connective tissue of the neck is also determined by incision. The gas burns with a pale bluish, almost colorless flame, a slight explosive sound being heard at the moment of ignition.

Peritoneal cavity dry and not distended with gas. Visceral and parietal peritoneum, including omentum, studded with small, gray, translucent, firm nodules (miliary tubercles), averaging 0.5 to 1 mm. in diameter. Old fibrous adhesions around the spleen and between liver and diaphragm. Diaphragm on right side at 5th, on left at 6th rib.

Pleural cavities completely obliterated by old adhesions. Pericardial sac contains 30 c. c. thin, blood stained serum containing gas bubbles. Dimensions of heart normal, save slight hypertrophy of right ventricle. Cardiac veins and arteries are full of gas. Right ventricle and auricle contain soft, dark red coagula and thin, lac-colored blood, with which are mingled gas bubbles in large number. The left cavities of the heart contain similar material in smaller quantity, together with gas bubbles. Pulmonary artery and veins contain gas bubbles. The muscular wall of the heart is soft and flabby and presents a peculiar spotted appearance on section. There are numerous small cavities, about 0.5 to 1 mm. in diameter, containing gas throughout the myocardium. Immediately around these cavities the muscular tissue has a pale whitish appearance. Cardiac valves normal, except a few whitish atheromatous patches on the aortic segment of the mitral valve. Thickness of wall of left ventricle 16 mm., of right ventricle 5 mm., length of ventricular cavity 7.5 cm., inner circumference of aorta just above valve 8 cm. Endocardium diffusely blood stained.

Ascending arch of aorta much dilated, wall thickened, inner surface diffusely blood stained and presenting many firm, partly calcified atheromatous patches. 5 cm. above the aortic valves, just above the duplicature of the pericardium, is a nearly circular opening 3.5 cm. in diameter, with sharply circumscribed, smooth, rounded edges in the anterior and right wall of the aorta. This opening, which is 2.5 cm. below the origin of the innominate artery, leads into a large aneurismal sac, which has developed chiefly forwards and to the right and produced the tumor visible externally. The aneurism measures 12 cm. in vertical and transverse diameters, and 7.5 cm. in anteroposterior diameter. It extends from the upper margin of the clavicle to the fourth rib. Its median border corresponds to the midline of the sternum. The eroded surfaces of the first, second and third ribs and of the sternum form a part of the anterior wall of the aneurism. The second rib has been completely destroyed at a point 3 cm. from the border of the sternum. The aneurismal sac is in relation above with the subclavian vein and innominate artery and vein, below with the pericardium, to the right with the upper lobe of the right lung, to which it is firmly adherent, and on the median side with the thickened tissues of the anterior mediastinum. There are no evidences of pressure upon the oesophagus, trachea, or main bronchi. The walls of the aneurism are intact, except at the two openings in the skin already described. The sac of the aneurism is completely filled with coagula, those nearest the mouth being soft, dark red post mortem clots; the rest, which nearly fill the cavity, are laminated gray and reddish firm ante mortem coagula, which can be removed with the fingers without much difficulty, except those nearest the wall, which are firmly adherent. The laminated coagula present pale white streaks and dots and contain many gas bubbles.

Lungs, hyperaemic, oedematous, pigmented with coal, in upper lobes old cavities with fibrous wall and caseous contents, dense interlacing fibrous tissue, a few caseous nodules, and throughout both lungs many small, firm, gray miliary tubercles, without fresh pneumonia. Bronchi contain mucopus, their inner surface diffusely reddened.

Spleen, dimensions 13 x 8 x 4 cm. Studded on its surface with miliary tubercles, with a few grayish tubercles in parenchyma, which is soft, brownish red, and on pressure permits a large number of gas bubbles to escape. Malpighian bodies indistinct.

Kidneys, dimensions normal, capsule not adherent, surface smooth, thickness of cortex 8 mm. Markings coarse, color pale, a few small, grayish yellow tubercles visible on the surface and on section. Gas bubbles escape on section and by pressure from the renal blood vessels. The tissues in the pelvis of kidneys are diffusely red, from imbibition of blood coloring matter.

In lower third of ileum are several scattered fresh tubercular ulcers, nearly circular in shape, not extending deeper than submucosa. Mesenteric glands pale, swollen, contain a few tubercles.

Stomach and duodenum contain much mucus. Dark, brownish yellow bile can be readily squeezed from gall-bladder through common bile-duct.

Liver, dimensions 29 x 19 x 7 cm., presents emphysematous crackling on pressure. Surface and cut section present a peculiar mottled appearance, from the presence of many little, round, gas-containing cavities, about 1 to 2 mm. in diameter, which appear transparent as seen through the capsule. Walls of cavities appear smooth and formed by pale whitish liver substance. Hepatic parenchyma soft, pale and brownish in color. Gas bubbles in large number in the vessels of the liver.

Urinary bladder contains 50 c. c. clear urine. Flat, depressed scar on glans penis in place of frenum. Old scars in groins.

Aorta very atheromatous throughout its course. Large and small arteries present scattered patches of atheroma. The intima of all the large arteries and veins is diffusely red, from imbibition of blood coloring matter. The blood is thin, watery, transparent and lac-colored, and everywhere contains gas bubbles. The gas bubbles are both large and small. No distinct odor can be detected from the blood, and in general there are no putrefactive odor and no greenish discoloration about the body.

Microscopical Examination of Fresh Blood and Organs.—The blood, taken with care from the interior of veins and arteries immediately after opening them, shows fewer and paler red blood corpuscles than normal. Many of the red corpuscles are irregular in shape, and there are shadows of corpuscles. There has evidently been a solution of some of the coloring matter of the red corpuscles. The leucocytes are swollen and their granules often in molecular movement.

The blood from the heart and vessels is rich in bacilli, about 3 to 5 μ. in length, about the thickness of anthrax bacilli, with ends slightly rounded, sometimes almost square-cut, occurring chiefly in pairs and in irregular masses and not in long chains. The same bacilli are present also in very large number in cover-slip preparations from the laminated clot of the aneurism, the liver, the spleen and the kidney. In all of these situations the bacilli appear to be in pure culture, no other species being observed. The bacilli are not motile. They stain readily with the usual aniline dyes, but often present small unstained spots. They possess a capsule, evident on the stained specimens.

Frozen sections of the fresh liver show the small cavities, visible to the naked eye, to be surrounded by the liver cells, much disintegrated and fatty degenerated. Here the hepatic cells lie loose, with an indefinite granular detritus between them, whereas elsewhere the rows of liver cells, which also contain large and small oil globules, are preserved. Bacilli are present throughout the liver, but are by far most abundant in and near the small cavities. Several fresh miliary tubercles are seen in the sections.

Sections of the frozen fresh myocardium show also little cavities with many bacilli and disintegrating muscular fibers containing fine fatty granules in their walls. Elsewhere the muscular fibers show good striation, with a few scattered small oil globules. There appear to be more fatty granules in the broken up fibers near the cavities than elsewhere.

Sections of the fresh kidney show with a low power areas darker and more opaque than the rest of the tissue and corresponding to groups of perhaps a dozen cortical tubules. In these areas the epithelial cells are finely fatty and desquamated, and here there are many bacilli, whereas elsewhere the epithelium does not appear much altered and bacilli are less numerous. A few small gas-containing cavities are present in the cortical substance. There are some atrophied glomeruli with thickened capsules, foci of increased intertubular tissue, thickened arteries, and many waxy casts in Henle's tubules.

Microscopical Examination of Hardened Organs.—Small pieces of the organs were hardened in alcohol. Sections of these confirm the observations made on the fresh tissues. The liver shows many miliary tubercles with giant cells. These tubercles tend to become fibrous rather than caseous. There is much bile pigment in the hepatic cells. There is a new growth of fibrous tissue in the external capsule of the liver. The interlobular connective tissue is moderately thickened, chiefly in irregular nodules and areas in connection with the tubercles. The kidney is the seat of chronic diffuse nephritis in moderate degree.

In the sections the bacilli stain readily with all of the ordinary aniline dyes (methylene blue, gentian-violet, fuchsine) and stain well even with haematoxylin. They stain excellently by Gram's method, retaining the color after the nuclei have been decolorized. Their length averages one-half to two-thirds the diameter of the red blood corpuscles seen on the same sections, and their thickness is perhaps a little greater than that of anthrax bacilli, from which they are especially distinguished by not occurring in long chains.

The bacilli are found abundantly in the large arteries and veins throughout the lumen, but seem to be more numerous in the veins. Some sections of vessels, particularly of arteries, are free from the bacilli. Although some capillary blood vessels are plugged full of bacilli or contain them in large number, most of the capillaries in the different organs do not contain them. The nuclei in the walls of the vessels containing bacilli, even those of the endothelium, stain well as a rule, but sometimes the nuclei do not stain. Occasionally the bacilli are found not only in the lumen, but also in the walls of the vessels, and they occur in scattered spots in the tissues. The lymphatics in the pericardium are beautifully injected with masses of bacilli.

When the bacilli are accumulated in large masses in the tissues the surrounding nuclei usually do not stain. These bacillar masses can be seen with the naked eye on stained specimens as deeply stained dots, and occur scattered irregularly. The zone of unstained nuclei around the clumps of bacilli in the tissues is wider in the liver than in the kidney, and wider in the kidney than in the heart muscle. Elsewhere the nuclei stain well. The absence of nuclear staining must be due to the products of the growth of the bacilli, for it occurs at a considerable distance, for instance the thickness of a lobule in the liver, from the spot where the bacilli are present. There is no nuclear fragmentation. The nuclei either cannot be made out at all or appear as very pale bodies. In the heart muscle near the masses of bacilli in the tissue the nuclei seem smaller than elsewhere, whereas in the liver there are many nuclei swollen to three or four times their normal size, appearing as empty cavities with a stained rim, suggesting somewhat drops of fat, from which, however, they can be readily distinguished by the sharp stained ring around them and the transitions to the normal nuclei. These peculiarly altered nuclei can be seen scattered irregularly through the liver as well as in or near the areas with unstained nuclei. There is absolutely no evidence of reaction in the tissues around these areas or the masses of bacilli.

The cavities which were visible to the naked eye and on frozen sections as gas blebs appear on the hardened sections. They vary in size and shape. The smaller ones are regularly round, often with smooth walls. Larger ones, which may be 1 to 2 mm. in diameter, may be oval or irregular in shape, and evidently may be formed by the coalescence of smaller cavities. The bacilli are in almost or quite continuous mass in the margin of these cavities, and for a variable distance around them the nuclei do not stain. There is evidence of pressure upon the tissues around the cavities. Areas can be seen in which dense masses of bacilli lie between the broken up loose tissue elements (liver cells, muscle-fibers) without stained nuclei. In spots in the liver the intralobular capillaries are plugged with bacilli. Exceptionally, bacilli are present in the lumen of urinary tubules. They are generally absent from the intertubular and glomerular capillaries, and are more abundant in the cortex than in the pyramids. The bacilli are much more numerous in the hepatic and portal veins than in the hepatic arteries.

Cultures in nutrient agar-agar were made from the heart's blood, the aneurismal clots, the liver, spleen and kidney. These cultures were made according to our usual custom in the progress of the autopsy. The surface of each organ as soon as exposed is thoroughly burnt over a sufficient area by a flat piece of metal heated to redness, and Nuttall's thick platinum needle[2] thrust into the interior through the burnt area. As it seemed probable that the bacilli found on the cover-slips would prove to be anaerobic, in addition to the ordinary roll cultures, tubes one-half or three-quarters filled with liquefied agar at 40° C. were inoculated with material from the platinum loop, and after thorough mixing the agar was allowed to solidify in the upright tubes. The tubes were placed in the thermostat at 37° C.

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[2] Nuttall. Centralblatt f. Bakteriologie, 1892, Bd. XI, No. 17, p. 538.

After 24 hours no growth appeared in any of the roll cultures and none subsequently developed. On the other hand the tubes of high agar showed an abundant growth in the form of small, grayish white colonies, with many gas bubbles, in the lower two-thirds of the agar, but no growth in the upper third. The organism in the cultures is therefore anaerobic and incapable of development in plain agar in the presence of much free oxygen. Microscopical examination of the cultures showed only one species, and that a bacillus identical morphologically with that found in the cover-slip preparations of the fresh blood and organs. The cultures from the blood and all the organs mentioned presented the same appearance and were of this same bacillus. From single colonies other tubes of high agar were inoculated, and thus the organism was obtained in pure culture for further study, the results of which will now be presented.

Morphology.—The usual form of the bacillus is straight, but sometimes in fresh cultures, more frequently in old ones, slightly curved or bent forms are seen. The ends of the bacillus are slightly rounded, or they may appear, especially the adjacent ends in pairs or short chains, as nearly or quite square-cut, but this latter appearance is not so uniform and outspoken as with the anthrax bacilli.

The bacillus varies somewhat in size, especially in length, in different culture media. It appears usually thicker and more variable in length in artificial cultures than in the blood of animals or of man. The average thickness in fresh cultures is a little greater than that of the anthrax bacillus. The average length in fresh cultures may be placed as 3 to 6 μ., but forms are common in the same culture which are shorter, as well as those which are two to three times longer.

The bacilli occur singly, in pairs, in clumps, and sometimes in short chains. When placed end to end the rods may be in a straight line, but they are often at an angle with each other. Long chains and long threads have been seen exceptionally both in the blood of animals and in cultures, but the absence of a tendency to grow in long chains is a distinguishing point between these bacilli and those of anthrax.

The bacillus is not motile. This point has been tested repeatedly both in the blood of animals and in fresh bouillon and agar cultures.

As has already been mentioned for the sections, the bacilli stain readily with ordinary aniline dyes. With Gram's method the dried and heated cover-glass preparations do not retain the dye so persistently as do the sections of alcohol-hardened specimens. With methylene blue the rods from fresh cultures stain uniformly, or they may show little unstained dots irregularly placed and occasionally so numerous as to give a granular or vacuolated appearance to the rods. Less frequently are also seen in some bacilli specks more deeply stained than the rest of the rod.

Capsules can often be demonstrated around the bacilli, but they do not seem to be constantly present. They make one of the most important morphological features of the bacillus. They have been found both in cultures and in the animal body. They appear as clear zones when unstained. We have found the following method well adapted for bringing to view capsules, which, however, may appear by ordinary methods of staining. The dried and not overheated cover-slip preparations are treated first with glacial acetic acid, which is allowed to drain off and is at once replaced by a strong aqueous solution of gentian-violet, which is to be added several times until it has taken the place of the acid. The specimen is then examined in the coloring solution, the thin layer of which left under the cover-glass after soaking up the excess with filter-paper not interfering with a clear field. The capsules are then very distinct, but often not in water or after mounting in balsam. The width of the capsule varies from one-half to twice the thickness of the bacillus, these differences depending on the exact method of treatment as well as on actual differences in thickness. The outer margin of the capsule is stained, leaving as a rule a clear zone immediately around the bacilli. We have been most successful in demonstrating the capsules in cultures in plain agar and in the blood of rabbits.

Various involution forms are found in old cultures. The most remarkable ones were observed in five per cent and ten per cent sugar gelatine cultures a month old. Here we observed long threads, sometimes reaching through several fields of the microscope (homogeneous immersion $\frac{1}{12}$), thin rods, thick rods, irregularly swollen, varicose rods, pear-shaped rods, exquisite spiral forms, curved and bent forms, rods with distinct capsules without special staining, and various irregularities in staining. Fresh cultures from these old ones were pure of the typical bacilli. In an agar culture four months old we observed also remarkable involution shapes, including some very small, coccus-like forms and capsules containing small stained particles or even empty. On the other hand a sugar bouillon culture of the same age was nearly free from involution forms, and a sealed tube containing an agar culture 89 days old was also nearly devoid of unusual forms.

The bacillus does not form spores.

Cultures.—The bacillus is capable of growth upon all of the ordinary culture media. It grows best at temperatures of 35°-37° C., but it grows also at ordinary room temperatures (18°-20° C.). It is anaerobic. No growth takes place in plain or in sugar bouillon without removing the oxygen. In solid media, gelatine and agar, inoculated as soon as solidified after thorough steaming, it grows in the deeper layers even when the air has free access to the tube. For making anaerobic cultures various procedures were adopted, but the one found most convenient, and which we used chiefly, was to place the tubes in narrow cylindrical jars (a little higher than the length of the test tubes) containing pyrogallic acid and ten per cent caustic potash solution, according to the method recommended by Buchner.[3] Although the oxygen is not as completely displaced by this method as by some others, it is sufficiently absorbed for our purpose. The stopper of the jar was sealed with a thick layer of paraffin.

Cultures in Nutrient Agar-Agar.—Gelatine and agar tubes were usually inoculated as soon as the medium was solidified after thorough steaming by which the air is temporarily displaced. For cultures in high agar or gelatine the tubes were filled one-third to one-half of their height with the medium. Stab cultures were made with a long platinum needle reaching to the bottom of the tube.

These cultures in ordinary neutral or alkaline nutrient agar to which the air has free access through the cotton plug show a good growth at the end of 24 hours at body temperature. The colonies in the lower part of the needle track are larger than those above, the uppermost ones being very minute. The growth reaches to a variable height beneath the surface. It usually ceases about 10 to 12 mm. below the surface when the tube has been inoculated after sufficiently prolonged steaming to displace all of the air. In other cases it may not come within 3 to 4 cm. of the surface. After artificial cultivation for several generations the growth seems to approach nearer the surface than at first. The colonies are grayish white to a more opaque white or even brownish white color by transmitted light, sometimes with a central darker dot, the opacity of the color depending upon the thickness of the colony. The largest colonies at the end of 24 hours do not exceed 0.5 to 1 mm. in diameter. They may subsequently attain a diameter of 2 to 3 mm. or even larger. They appear as spheres or ovals, generally more or less flattened, with usually irregular contours, the irregularity being due to little projections or prongs from the surface of the colonies, these irregularities being more evident with a loop than with the naked eye. The colonies may appear as little irregular masses with projections. After several days or weeks, single, well separated colonies may attain a large size and be surrounded with projections either in the form of little knobs or spikes or of fine branching hair-like or feathery processes. In the former case the colonies present a mulberry or thornapple appearance; in the latter they may be compared to spherical white thistleballs or powder puffs. Such colonies in old cultures sometimes reach 1 cm. in diameter. Similar feathery projections may be present in places when the stab growth is continuous.

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[3] Buchner: Centralblatt f. Bakteriologie, 1888, Bd. IV, p. 149.

Bubbles of gas make their appearance in plain agar as well as in sugar agar, although less abundantly in the former, and sometimes not appearing until after 24 hours. These bubbles first appear in the line of growth, but they are soon present throughout the agar, often at a distance from the actual growth. Fluid is pressed out into the spaces occupied by the bubbles as well as upon the surface of the agar, anl this fluid may be turbid from the presence of bacilli. The production of gas is less abundant at room temperatures than at that of the thermostat (35°-37° C.). There is usually no very distinctive odor to be appreciated from the cultures in gelatine or agar, but there is an odor not easily described, not putrescent, something like that of stale glue, and this odor is in some cultures more pronounced than in others. It was sometimes noticed that when the agar cultures were thoroughly crushed in a little bouillon a foul odor can be detected.

Nutrient agar containing one per cent glucose permits a much more rapid and luxuriant growth and especially more abundant and speedy gas formation than does plain nutrient agar. Here the growth in high layer comes nearer the surface, often within 2 to 3 mm. when the air has free access to the tube through the cotton plug. Neutral or slightly alkaline media were employed, and these were turned strongly acid by the growth of the bacillus. In anaerobic cultures in sugar agar made according to Buchner's method the production of gas is so great as often to split the agar and force it into the upper part of the tube. In these anaerobic cultures there is of course less difference between the size of the colonies in the lower and those in the upper layers, and the growth usually comes to the surface, but does not spread out there. Generally an excessive quantity of fluid accumulates on the surface, and this is turbid from the presence of the bacilli. The agar is not liquefied by the growth of the bacilli.

Cultures in Nutrient Gelatine.—The bacillus grows in ordinary neutral or alkaline nutrient gelatine, but not so well as in that containing glucose. Gas is formed in both media, but much more abundantly in sugar gelatine. Five per cent gelatine is much better than 10 per cent gelatine for the development of the bacillus. The bacillus is best classed among the nonliquefiers of gelatine, although in anaerobic cultures in 5 per cent sugar gelatine there may be a slight softening due to peptonization of the gelatine over a limited area, as is made manifest by the settling of the growth toward the bottom of the line of puncture in stab cultures, and by slight displacement of the gas bubbles in changing the position of the tube. Cultures in gelatine which have developed at 35°-37° C. become solid upon cooling the tube. In gelatine cultures at 20°-23° C. in high layer to which the air has free access through the cotton plug, the growth occupies the lower third to one-half of the medium, not reaching as near the surface as in agar cultures at body temperature. The appearance of the colonies in gelatine does not differ especially from that of those in agar.

Cultures in Bouillon.—We have not noticed any development of the organisms in tubes of bouillon to which the air has free access, but the growth is abundant in anaerobic cultures in both plain and sugar bouillon. In sugar bouillon there is very abundant gas formation, small bubbles accumulating on the surface so as to form often a foamy layer. As soon as the development of the organism begins it goes on with extreme rapidity, so that clear bouillon may become very cloudy with an abundant sediment in two or three hours. The development in sugar bouillon is well marked in Buchner's jars in less than 18 hours. The diffuse cloudiness of the bouillon gives place to a clearer or even an absolutely clear and transparent appearance in the course of a few days by the settling of the sediment. This sediment is abundant, white, uniform or sometimes flaky. Upon shaking it floats up in viscid threads or clouds or, if in flakes, can be readily broken up so as to mingle uniformly with the fluid and produce diffuse cloudiness, or it may be in the form of little specks less easily disintegrated by shaking and quickly settling again upon standing. The reaction of the sugar bouillon is of course strongly acid after the development is completed. The odor of bouillon cultures is like that of agar cultures and may be compared to sour stale glue. It is not a distinctly putrescent odor.

Cultures in Milk.—Growth is speedy and abundant in anaerobic milk tubes at body temperature, but was not observed in tubes exposed to the air. In 24 to 48 hours the milk is coagulated, the clot being either uniform or firm, retracted and furrowed with the marks of gas bubbles which develop abundantly. In milk colored with blue litmus, which is of course completely decolorized simply by absorption of oxygen in anaerobic cultures, the coagulated milk becomes deep pink upon exposure to the air.

Cultures upon Potato.—In potato tubes prepared according to Bolton's method[4] and put in the Buchner jars and kept at body temperature, abundant gas formation occurs in 24 hours in the fluid accumulated around the bottom and sides of the potato, which thus becomes surrounded with a frothy fluid. After complete absorption of the oxygen there appears a thin, moist, grayish white growth on the surface of the potato.

The bacillus grows well with formation of gas bubbles in ascitic fluid in anaerobic culture.

Vitality.—As already mentioned, the bacillus forms no spores. The vitality of the cultures is of very variable duration, according to the character of the medium and the mode of cultivation. Cultures in sugar bouillon in Liborius tubes in which the oxygen was replaced by hydrogen we found to be dead in three days and they may die sooner. On the other hand we found sugar bouillon culture kept for 123 days in Buchner's jar at body temperature to be still living. The same was true of a culture in plain agar. Cultures in sugar agar are shorter-lived than those in plain agar and may be dead in 28 days or even less. A culture in plain agar exposed all of the time to the free entrance of air through the cotton stopper had a few living bacilli at the end of 84 days, although most of the organisms were dead. Cultures in sugar gelatine appear to be longer-lived than those in sugar agar, and cultures kept in Buchner's jar longer-lived than those to which the air can enter. There were curious and not readily explained differences in the vitality of the cultures, some being still alive which were under apparently the same conditions and of the same age as those which proved to be dead. A convenient way of keeping the cultures alive for a long time is to seal the agar tubes hermetically after two or three days' growth.

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[4] Bolton: The Medical News, 1887, Vol. I, p. 318.

Thermal Death-Point.—This was tested according to Sternberg's method[6] on fresh bouillon cultures. These were killed by exposure for ten minutes to a temperature of 58° C. They were not completely killed by the same temperature after five minutes.

Inoculation of Animals.—Quantities up to two and one-half cubic centimeters of fresh cultures in sugar bouillon and of suspensions in salt solution or bouillon of fresh agar cultures were inoculated into the ear veins of rabbits without any manifest symptoms, the animals surviving. Larger quantities were not employed. In only one instance was a rabbit killed by intravenous inoculation, and this case will be described later. We cannot therefore consider this bacillus as pathogenic for healthy rabbits under ordinary conditions.

If, however, the animal be killed after the intravenous injection of the bacilli, then these develop in the vessels, tissues and organs with abundant formation of gas. Upon this point we have made twenty experiments upon rabbits and one upon a dog. The animals were killed at a variable period after the injection. As the results of these experiments were uniform and conclusive, it is necessary to give only a few of the protocols as examples.

Experiment 1.—Large rabbit. 1 c. c. sugar bouillon culture (the ninth generation from the original case) 24 hours old, which had developed in Buchner's jar at 36° C., showing abundant growth with gas production, was injected into the ear vein and immediately after the injection the rabbit was killed by a sharp blow at the back of the neck. The animal was kept in a room the temperature of which was about 18°-20° C. A control rabbit was killed and kept under the same conditions. At the end of 18 hours emphysematous crackling could be felt in the groins of the inoculated rabbit and elsewhere. The femoral vessels were now exposed and found to be full of gas bubbles. The autopsy was then made. Peritoneal cavity full of gas. The heart cavities contain soft, dark coagula with lac-colored blood and many gas bubbles. The blood vessels, both arteries and veins, are full of gas bubbles. The abdominal vena cava and other abdominal veins are blown up with a continuous volume of gas. The inner coat of blood vessels is diffusely red from inhibition of blood coloring matter. The liver is soft, pale and crackling. It is studded with little cavities containing gas. The spleen is soft, crackling and swollen with gas bubbles. The kidney also contains gas. The tissues around the kidney, the retroperitoneal tissue, the tissues in the groin, over the abdomen, are emphysematous. The gas burns with a bluish flame, and upon ignition a slight explosive sound is heard. Blood stained fluid in the pericardium and pleura contains gas bubbles. No odor of putrefaction. Cover-slip preparations from the blood vessels, liver and spleen show an enormous number of bacilli, like those injected, provided with beautiful capsules easily demonstrable by the acetic acid method. No other species of bacteria are observed. Anaerobic cultures and cultures in high liquefied agar from the blood and spleen prove to be pure cultures of the typical bacilli.

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[6] Sternberg: Am. Journn. of the Med. Sciences, July, 1887, p. 146.

The control rabbit shows no development of gas in the vessels or organs. No bacteria are found in the blood, and cultures from the blood are negative.

Experiment 2.—Full-grown rabbit. 0.5 c. c. 24-hour anaerobic sugar bouillon culture (13th generation) into ear vein. Killed after 5 minutes. Kept for 24 hours under same conditions as the preceding rabbit. At the end of this period animal much swollen, abdomen distended and tense, emphysematous crackling of subcutaneous tissues. Gas abundant in heart and blood vessels, serous cavities, spleen, liver and other parts. Bacilli present in large number. Cultures from heart's blood pure of the typical bacilli. Control rabbit free from gas formation in vessels.

Experiment 3.—Large rabbit. 1 c. c. of the same culture as that used for preceding rabbit injected into ear vein. Killed after one hour. The inoculated ear cut off near the base and remaining cut surface burnt. Result as in preceding case, but the animal not so much swollen from gas formation.

When the animal is killed shortly after the injection into the circulation and is then kept at temperatures between 30° and 35° C. the development of bacilli and gas goes on with almost startling rapidity, as is illustrated by the following cases:

Experiment 4.—Medium sized rabbit. 1 c. c. anaerobic sugar bouillon culture (17th generation), 24 hours old, into ear vein. Killed after two minutes and immediately placed in incubator regulated accurately at 32° C. Autopsy made 6 hours after death. Abdomen much distended. Emphysematous crackling over thorax, abdomen and inside of thighs. On incision, gas, which burns with a blue flame, escapes freely from the abdominal cavity. Fundus of stomach digested. Vena cava inferior and other abdominal veins appear to contain no blood, but to be blown up with a continuous volume of gas. Abdominal aorta contains gas-bubbles and a little blood, but is not distended. The heart cavities and blood vessels everywhere contain gas. The liver is completely filled with small and larger gas bubbles, forming a pale, soft emphysematous pulpy mass. Many of the gas blebs at the surface have ruptured. Spleen full of gas blebs. Gas in kidney. Muscles of thigh and elsewhere streaked with whitish lines containing gas. Pleural and pericardial sacs full of gas. Emphysema of subpleural and subperitoneal tissue. Urinary bladder free from gas. Blood lac-colored, with soft dark clots. Typical capsule-staining bacilli in enormous number in the blood, liver, etc. No other species observed. Cultures from the blood are pure of the bacillus injected.

A control rabbit killed at the same time and kept in the incubator was examined after 8¼ hours. It presented beginning greenish discoloration of the abdominal walls. No gas in the heart, blood vessels, liver or serous cavities. A few bacteria in the portal vein, none found elsewhere in the blood. Cultures from the blood and organs sterile.

This case shows that in the short space of 6 hours after death gas and bacilli have developed at the temperature of 32° C. enormously, and to a greater degree than is observed after 24 hours at ordinary room temperatures.

Experiment 5.—Large rabbit. 1 c. c. anaerobic sugar bouillon culture (19th generation), 48 hours old, into ear vein. Killed after one minute by blow at back of neck, kept in incubator at 30° C. Examined after four hours. No subcutaneous emphysema. Gas-bubbles in femoral vessels. Gas and frothy reddish serum in moderate amount in peritoneal cavity. Stomach not digested. Vena cava abdominalis distended with large gas-bubbles mixed with dark blood. Smaller gas bubbles in abdominal aorta. Arteries and veins generally contain gas. Gas more abundant in right than in left cavities of heart, in pulmonary artery than in pulmonary veins. Liver contains many gas blebs. Gall-bladder distended with gas. Gas-blebs in spleen, gas in pleural cavities, in diaphragm, in subserous tissues. Blood shows under microscope distorted and pale red blood corpuscles, yellow plasma and leucocytes swollen, presenting molecular movement of granules and round apparently empty nuclei. Typical bacilli in pure culture in blood and elsewhere.

This case shows that at the temperature of 30° C. the bacilli and gas have developed to a marked degree within 4 hours after death, the animal having been killed one minute after intravenous injection of 1 c. c. bouillon culture. The dead animal body evidently offers the most favorable conditions possible for the growth of the bacillus. This growth is many times more rapid at warm temperatures than at temperatures below 20° C.

Several experiments were made to determine the difference in time required for the development of the bacilli with formation of gas throughout the blood vessels when the organisms are introduced only at one point in the vascular system after death and when they are distributed, as in the preceding experiments, just before death throughout the circulation.

Experiment 6.—Large rabbit. Killed by chloroform. Four hours after death a small window was cut in the thorax, the surface of the left ventricle singed with a hot knife, and two loopfuls from a 72-hour anaerobic agar culture (7th generation) thrust into the left ventricle. The rabbit was kept in the room at a temperature of 18° to 20° C. After 24 hours the femoral vessels were exposed and found to be free from gas. After 48 hours gas was found in the femoral artery, but none in the vein. The autopsy was then made. Gas bubbles quite abundant in all of the arteries, also in both sides of the heart, pulmonary vein, pulmonary artery and venae cavae near the heart, but none was found in the veins elsewhere, and none in the liver, spleen or connective tissue. Cover-slip preparations from the heart and aorta show the characteristic capsulated bacilli in large number, but no bacilli are found in the abdominal vena cava, the femoral vein or the liver. Cultures from the blood in the thoracic aorta are pure of the same bacillus, those from the abdominal vena cava are sterile.

In this case, therefore, at room temperature the bacilli after 48 hours had not made their way into the veins except those near the heart, but had grown throughout the arteries; whereas after 18 hours in experiment 1, in which the bacilli had been introduced into the circulation just before death and the rabbit had been kept under the same conditions, all of the vessels and organs and serous cavities were full of gas and bacilli.

In the following experiment the culture was introduced into the right ventricle shortly after death and the animal was kept at the temperature of 32° C.

Experiment 7.—Rabbit killed by blow at back of neck. One-half hour after death heart exposed, and after singeing the surface, 0.5 c. c. of the same sugar bouillon culture as that used in experiment 4 injected with fine hypodermic needle into right ventricle, no air being permitted to enter. This rabbit was kept together with that of experiment 4 and a control rabbit in the incubator at 32° C. and was examined after 7½ hours. No subcutaneous emphysema. No gas in femoral artery or vein. No gas in peritoneal cavity, liver, spleen or kidney. Stomach not digested. Gas bubbles in abdominal vena cava, renal, portal, hepatic veins, in veins near the heart, in both sides of heart, pulmonary artery, pulmonary veins and thoracic aorta, none in abdominal aorta. The gas is most abundant in vessels leading to or from the right heart, and is in comparatively small amount in the vena cava in the abdomen. Cover-slip preparations show numerous typical bacilli in pure culture wherever gas is present. The bacilli are abundant in a smear preparation from surface of lung. None are found in the abdominal aorta. (The control rabbit of experiment 4 served also as the control for this experiment.)

If the result of this experiment be compared with that of experiments 4 and 5 in which the autopsies were made after 6 and 4 hours respectively, it is evident that it requires a much longer time even at high temperatures for the bacilli and gas to develop throughout the vessels and organs when they are introduced at one point after death than when they have been distributed throughout the circulation just before death, a result which of course might have been predicted

The last two experiments are also interesting as showing that the bacilli first grow in the course of the circulatory channels into which they have been introduced, in experiment 6 along the arteries and in experiment 7 along the veins, and in both cases through the pulmonary circulation.

Of all of the organs of the body the liver appears to offer the most favorable conditions for the rapid development of the bacillus after death and the formation of gas. Some of our observations seem to indicate that the bacilli develop sooner in the veins than in the arteries, but our attention was not directed sufficiently to this point to justify a more positive statement.

The length of time that the bacilli injected into the circulation may survive in the living body we have not accurately determined, but they may persist for at least 48 hours and probably longer. It requires a longer time for the bacilli and gas to appear throughout the vessels when the animal is killed several hours up to two days after the injection than when it is killed immediately. These points are shown by the following experiments:

Experiment 8.—Large rabbit. 1 c. c. 48-hour sugar bouillon culture (16th generation) in ear vein. Killed after 48 hours by blow on back of neck. Kept for five hours at temperature of 32° C. and then at temperature of 20° C. Autopsy after 32 hours. Animal enormously swollen. Subcutaneous emphysema. Abdomen distended with gas. Vessels and liver full of gas. Enormous number of typical bacilli in blood and organs. Cultures from blood pure of the characteristic bacilli.

Out of six experiments upon rabbits to determine the pathogenic effects of the bacillus, only one died from the effects of the inoculation, and this afforded an interesting result.

Experiment 9.—Pregnant rabbit. 1 c. c. sugar bouillon culture (9th generation), 48 hours old, into ear vein. Rabbit was found dead 21 hours after the inoculation, the body being still warm, although stiff. It was alive one-half hour previously and apparently in good condition. The autopsy was made 6 hours after death. By this time the body, which has been in a room at the temperature of about 18° C., is much swollen, the abdomen distended, and emphysematous crackling can be felt over the abdomen and the lower part of the thorax. Bloody fluid containing gas bubbles oozes from the vagina. On opening peritoneal cavity gas escapes freely, which burns with a colorless flame, a slight explosive sound being heard upon first applying the match. The cavity contains blood-stained fluid. Blood vessels, both veins and arteries, contain gas. The heart cavities on both sides contain soft dark clots with gas-bubbles. Spleen is somewhat enlarged, soft and dark red. Liver does not contain gas blebs, but gas is present in hepatic vessels. The inferior vena cava is blown up with gas. The uterus contains 6 embryos about 3 cm. long. The dilatations of the uterus corresponding to these embryos are greatly distended with gas. Two of the sacs are more distended than the rest, their walls are very thin, contain gas bubbles, and look as if they were just ready to burst. The embryos in these two sacs are macerated, dark livid red, partly destroyed and smaller than the rest, which are intact. The placentae contain gas bubbles in large amount.

Cover-slip preparations show an enormous number of characteristic capsulated bacilli in the distended uterine sacs, a large but not so great number in the liver, spleen, heart and blood vessels. Cultures from the uterus, spleen and left ventricle are pure of the typical gas-forming bacillus which was used for the injection.

The preceding case is instructive as showing that under special conditions the injection of cultures of the gas bacillus may prove fatal. The special condition existing in this rabbit and not in any of the others used for these experiments was pregnancy. It seems probable from the autopsy that two of the embryos in the uterus were already dead when the injection was made, and that in these embryos and the part of the uterus containing them the bacilli were able to gain a foothold and develop.

The case is especially suggestive in view of the number of cases which have been reported of death from supposed entrance of air into the uterine veins after abortions and injections into the uterine cavity. It will be noted that the autopsy was made in six hours after the death of the animal, and that already the vessels and peritoneal cavity were full of gas and that there was subcutaneous emphysema, a condition not found when the animal is killed immediately after the injection of the cultures, kept at room temperature, and examined at that short period after death.

Conclusions from the Experiments.—Our experiments, although they have not settled all of the points which suggest themselves as to the relation between our gas-producing bacillus and the animal body, have settled the principal questions which we had in mind when we began the experiments. We may draw the following conclusions from these experiments on animals.

The bacillus is not pathogenic under ordinary conditions for healthy rabbits in doses up to 2.5 c. c. of fresh bouillon cultures. Doses of one cubic centimeter may, however, under especial conditions, prove fatal, the special condition in our experiment being pregnancy probably associated with the death of two embryos either before or soon after the injection.

The bacillus develops rapidly in the blood after death, with formation of gas.

If the animal be killed immediately or soon after the intravenous injection of 0.5 to 1 c. c. bouillon culture, after 18 to 24 hours at the temperature of 18°-20° C. and after 4 to 6 hours at temperatures from 30° to 35° C. the bacilli are found abundantly, together with great formation of gas in the blood vessels and organs.

When the animal is killed several hours up to two days after the intravenous injection it takes a longer time before the bacilli develop throughout the vessels and tissues with gas formation, than when the animal is killed at once after the injection.

When the bacilli are introduced at one place in the vascular system soon after death, they develop in the course of the vessels into which they are introduced, and the time required for them to appear with gas formation throughout the vessels is at least two or three times as long as when the bacilli have been distributed throughout the circulation just before death.

It is noteworthy that in the instance in which the pregnant rabbit died from the effects of the inoculation death was sudden, and that within six hours after death the vessels contained gas and bacilli in large amount.

All of the conditions relating to the bacilli and their production of gas which existed at the post mortem examination of the patient J. M. were produced experimentally in animals by inoculation of pure cultures of the bacillus isolated from the body of the patient.

It is our intention to continue the experiments in order to determine more accurately how long the bacilli may survive in the body of a living animal, how they are disposed of there, and the possibilities of their exerting pathogenic effects under certain circumstances.

In endeavoring to select a name suitable for the bacillus described in this article we have thought of several designations, such as the following: *Bacillus aerogenes capsulatus, Bacillus sanguinis aerogenes, Bacillus aerogenes, Bacillus aerogenes cadaveris, Bacillus pneumathaemiae.* (The name pneumathaemia was introduced by Cless to designate the presence of air in the blood.) Upon the whole we prefer the first name, *Bacillus aerogenes capsulatus.* The presence of a capsule does not appear to be constant, but it is common and forms a characteristic feature of the morphology of the bacillus.

We have not been able to identify the bacillus with any hitherto described, but the descriptions of some of the gas-producing bacilli have been so meagre and unsatisfactory that it is possible that others have found the same organism. We find indeed records in our laboratory notes of encountering from various sources obligatory anaerobic gas-producing non-motile bacilli, some of which may have been identical with the present one, but these organisms were not studied sufficiently to establish their identity and the cultures have not been kept. There is a note of finding such bacillus in the human lung at autopsy, but there was no gas production in the body. We think it likely that our *Bacillus aerogenes capsulatus* is not an uncommon species. The bacillus described by E. Fränkel[6] in a case of gastritis emphysematosa may have been identical with ours, but it was studied only on hardened specimens of the stomach. There may of course also be other bacteria capable of producing similar effects in the animal body.

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[6] E. Fränkel: Virchow's Archiv, Bd. 118, p. 526, 1889.

We will now review briefly the main points in the case which formed the starting point of our investigations, and consider the bearing of the case upon other similar ones recorded in medical literature.

A patient with chronic pulmonary tuberculosis, acute miliary tuberculosis and a large sacculated aneurism of the ascending arch of the aorta, which had ruptured in two places through the anterior thoracic walls, died suddenly after repeated copious haemorrhages from the aneurism, but not immediately after the loss of blood. The autopsy was made in cool weather *eight hours after death* while the body was still warm, there being no odor or evidence of ordinary cadaveric decomposition present. The heart and blood vessels everywhere were found to contain gas bubbles in large amount; gas was also present in the subcutaneous connective tissue in some places, in the heart-muscle, liver and other organs. There was a solution of the blood coloring matter evidenced by the color of the blood, the diffuse red staining of the inner coat of the vessels and of the tissues in the pelvis of the kidney. The bacterioscopic examination of the blood revealed the presence of nonmotile, capsulated bacilli in very large number wherever gas was found, and no other species of microorganism could be detected. This bacillus was isolated in pure culture and found to be an obligatory anaerobe. Its morphological and biological properties were studied and have been described. An exact reproduction in every particular of the condition in the body relating to the presence of gas and changes caused by the bacillus was obtained by the injection of pure cultures of the bacillus into the circulation of rabbits shortly before killing the animal. No accurate determination of the gases produced by the growth of the bacillus was made save to establish by ignition the presence of hydrogen both in the original case and in the experimental animals.

There can be no doubt that the gas found in the vessels and organs at the autopsy on the patient was not atmospheric air, but was produced by the growth of the bacilli. The questions which at once suggest themselves are: Were these bacilli distributed throughout the circulation before the death of the patient? At what point did they enter the circulation? Were they concerned in causing death? An unequivocal answer cannot be given to these questions.

As regards the first question, the experiments upon animals speak strongly in favor of the view that the bacilli had entered the circulation before death. When these bacilli were introduced into the heart of the rabbit after death, it required so much longer for them to spread throughout the vessels and organs with gas formation as compared with their development when the bacilli were previously carried throughout the body by the circulation, that it seems doubtful that in the short space of eight hours, the body being kept in a cool place, the bacilli starting at one point could have developed so extensively and have produced so much gas throughout the vessels and organs as were found at the autopsy on the patient. Even when the animal was kept from the time of death at a temperature of 32° C. the bacilli which had been introduced after death into the right ventricle had not made their appearance in the abdominal aorta or the peripheral veins or produced gas blebs in the liver after 7½ hours (experiment 7). On the other hand we have experimental evidence of quite as rapid post mortem development of bacilli and gas as that occurring in the patient J. M. when the organisms have been previously introduced into the circulation. It may, however, be urged that special conditions favoring the growth of the bacilli, such as the disappearance of oxygen and possibly of other restraining influences of the fluids and cells, may have appeared in the anaemic body of the tuberculous patient at an earlier period after death than they do in the previously healthy animal.

Regarding the point of entrance of the bacilli it seems to us probable that they entered the aneurismal sac through the external openings in the chest wall. The sac of the large aneurism was nearly filled with laminated antemortem coagula. The conditions would seem to be suitable for the development of the bacilli in this thick clot, in the deeper parts of which we may suppose the oxygen to be in so small an amount as to permit the growth of the bacillus. The excellent development of the organism in fluid cultures with Buchner's method of anaerobic culture before there has been time for complete absorption of the oxygen, and the height to which it grows in solid agar cultures, indicate that it is not so susceptible to the restraining influence of a small amount of oxygen as are some strictly anaerobic organisms, and we believe therefore that it could find conditions for its growth in the thick clot of the aneurism, which was virtually shut off from the circulation, as well as in high layers of gelatine and agar exposed to the air. The aneurismal clot was found at the autopsy to be riddled with gas and discolored around the gas and to contain in enormous number the bacilli, in apparently pure culture. There was no other place in the body where equally favorable conditions for the growth of the organism were present unless it be in the intestinal canal, and the possibility of the entrance of the bacilli from the latter source cannot be absolutely excluded.

If, as it seems quite possible, the bacilli had entered through the ulcerated openings the clots of the aneurism and effected growth there before death, then the conditions seem to have been such as to permit the escape of gas into the circulation during life, the bacilli being also carried in just as they are carried to the surface of agar cultures by the escaping gas. The reduction of the blood pressure by the extremely exhausted and anaemic state of the patient would be a factor favoring the escape of the gas into the circulation. The splitting and elevation of masses of agar in our cultures show that the developing gas is capable of exerting considerable pressure. It seems to us therefore by no means impossible that the entrance of gas and of bacilli into the circulation from the aneurism may have been concerned in the death of the patient, which, as already stated, was sudden. Nevertheless we would express ourselves with reserve on these points. There were present other conditions sufficient to cause death, even sudden death; but in the light of experiments upon animals, all of the circumstances of the case seem best explained upon the supposition that the bacilli had begun to grow in the aneurismal clot and were carried into the circulation before death.

The experiments upon animals show that the bacilli are incapable of development in the circulating blood during life, as might have been predicted from the anaerobic character of the organism. If, however, the bacilli should find access to dead tissue, old fibrinous clots, cavities such as the intestine or uterus, under conditions where the amount of free oxygen is reduced to a minimum, there they might grow; and if the places where they develop communicate with the circulatory channels, then the gas produced and with it the bacilli might enter the circulation.

As is well known a large number of cases have been reported during the last seventy years of death in human beings attributed to the entrance of air into the veins, and a large amount of experimental work has been done both before and since this period in regard to the effects of the entrance of air into the circulation.

The experiments have shown that the sudden introduction of a large amount of air into the veins of rabbits and dogs is fatal, the lethal quantity for dogs when rapidly injected into the jugular vein varying according to Nysten[7] from 40 to 120 c. c., according to the size of the dog. Smaller quantities than this have, however, proved fatal to dogs. Hare,[8] who also found that quantities short of 40 c. c. did not kill dogs, believes that no such amount of air as would cause death in human beings can enter a vein injured by the surgeon's knife. While it is true that many of the cases reported as deaths due to entrance of air into veins opened by the surgeon are unsupported by any satisfactory evidence that this was the cause of death, still we cannot agree with Hare that all of the reported cases are of this character. The evidence seems to us conclusive in some of the cases that death really resulted from the sudden entrance of air.

When the air is introduced slowly and at intervals, enormous quantities can be injected in a comparatively short time without manifest injury. Thus

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[7] Nysten: Recherches de physiologie et de chimie pathologiques. Paris, 1811.

[8] Hare: Therapeutic Gazette, Sept. 16. 1889.

Laborde and Muron[9] injected into the external jugular vein of a dog 1120 c. c. in the space of one hour and a half without causing death, and Jürgensen[10] injected into the left femoral artery of a narcotized dog weighing 43.5 kilo. 3650 c. c. in the space of two hours and twenty-five minutes with only slight disturbance of the respiration and of the action of the heart. Under these circumstances the air bubbles pass through the capillaries and circulate with blood, but of course with such enormous quantities much of the air must be speedily eliminated.

The reported cases in human beings in which death has been attributed to the entrance of air into the circulation may be brought for the most part, although not wholly, into the following classes: surgical operations, especially about the neck, shoulder and skull; cases in which the air has entered the uterine veins, chiefly from the puerperal uterus, either spontaneously as after attempted abortions, or after injections into the uterine cavity; cases in which the air has entered from the stomach or intestine, and cases in which no point of entrance for the air could be detected.

We have already expressed our belief in the credibility of some in the first class of cases. We see also no reason to question a similar interpretation of such a case as that reported by Vogel,[11] in which a caseous lymphatic gland had brought about a communication between the right subclavian vein and a large bronchus. Death was instantaneous, and at the autopsy large air bubbles were found in the blood and in different abdominal organs.

The evidence is also strongly in favor if the view that some of the sudden deaths in the puerperal state which have been attributed to the entrance of air into the uterine sinuses, especially after injections of fluid and air into the cavity of the uterus, are in fact due to the assigned cause.

In 1882 Jürgensen[12] reported a case of sudden death of a patient with gastric ulcer which had bled profusely shortly before death. At the autopsy 22 hours after death gas was found in the blood vessels, the peritoneal cavity, the pericardial sac, the subperitoneal tissue, the spleen and the liver, which was emphysematous. In the floor of the ulcer, which had not perforated into the peritoneal cavity, was found an open vein, probably the splenic, 3 to 4 mm. in diameter. Jürgensen believes that the air found post mortem in the vessels entered the circulation during life through the open vein in the floor of the ulcer, passed through the portal capillaries into the hepatic veins to the inferior vena cava and the right heart, thence through the pulmonary circulation to the left heart and the systemic circulation. This interpretation seems to us altogether improbable, and one of us[13] in 1885 suggested that in this case the gas developed in the vessels after death, attention being called to the presence of gas elsewhere than in the blood vessels in this case, a point which Jürgensen does not consider in his first report. In 1887 Jürgensen[14] reported another case occurring a few months after the first, in which a gastric ulcer had perforated into the peritoneal cavity. At the autopsy, 22 hours after death, gas was found in the peritoneal and pleural cavities, the heart, arteries and veins, and the subcutaneous tissues of the abdomen. There was beginning green discoloration of the abdominal walls. No vessels with ulcerated openings were found in the ulcer or elsewhere. This case is interpreted by Jürgensen in an entirely different manner from the first, the gas in the vessels as well as that in the serous cavities and connective tissue being attributed to a gas-generating something, presumably a microbe, but he thinks the gas was developed in the circulation partly during life because just before death the jugular veins on the right side of the neck were observed to swell up, a phenomenon noted also in the first case. In the light of our case and of the investigations reported in this article these cases are open to a clear and satisfactory interpretation without recourse to the adventurous hypothesis advanced by Jürgensen in his first article.

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[9] Laborde and Muron. Comptes rendus de la Soc. de Biologie, 1873, t. V.

[10] Jürgensen: Deutsches Archiv f. klin. Med., Bd. 31, p. 458, 1882.

[11] Vogel: Berliner klin. Wochenschrift, 1882, No. 12.

[12] Jürgensen: *Loc. cit.*

A considerable number of cases are to be found in medical literature in which gas, which could not be explained as due to ordinary cadaveric decomposition, was found in the blood vessels after death, without any opening through which air could enter the circulation. In some the death was sudden, and no satisfactory cause of death could be found except the gas in the blood. Some of these cases are very curious, as will be seen by those who consult the works of Cless[15] and of Couty.[16] Most writers dismiss these cases as unworthy of credence, or as referable to ordinary post mortem decomposition, or as attributable to the entrance of air during the post mortem examination. Some of the older writers and even modern ones have seriously discussed the possibility of the spontaneous generation of air or gas in the circulating blood during life as an explanation of these cases. Ewald and Kobert[17] believe that they have found the key to their solution by their demonstration that the lungs during life are not air-tight, but apparently without rupture permit under pressure air to be forced through which makes its appearance in the pleural cavities and in the blood of the heart and elsewhere. Our observations reported in this article suggest an explanation of some of these cases more satisfactory than those hitherto offered.

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[13] Welch: A System of Practical Medicine by American Authors, edited by William Pepper. Vol. II, p. 510. Philadelphia, 1885.

[14] Jürgensen: Deutsches Archiv f. klin. Med., Bd. 41, p. 569, 1887.

[15] Cless: Luft im Blute. Stuttgart, 1854.

[16] Couty: Etude expérimentale sur l'entrée de l'air dans les veines et les gas intravasculaires. Thèse, Paris, 1875.

[17] Ewald and Kobert: Pflüger's Archiv f. d. gesammte Physiologie, 1883, Bd. 31, p. 182.

Fischer,[18] in his well known paper on the dangers of the entrance of air into the veins, justly says: "The weakest part of the entire doctrine regarding entrance of air into the veins of human beings during an operation is the extremely variable and undemonstrative character of the recorded autopsies." Although he does not say so, he evidently quotes from Greene's article,[19] which is the best consideration of the literature of the subject which has come to our notice in the English language up to the date of its publication (1864), in giving the number of autopsies as only eighteen and in stating that in only six of these is there any statement as to the length of time between death and the post mortem examination, in five of these cases the time being 18 to 26 hours and in one 52 hours.

May reports a case of sudden death in the puerperal state in which the autopsy was made 6 hours after death, and in which he considers that the presence of air in the vessels was the only cause to which death could be attributed.[20] At the other extreme is a case for which the same explanation is accepted and in which the vessels were examined for the presence of gas five days after death.

Any one who will examine with critical spirit the reported cases of death from the entrance of air into the vessels will be impressed with the unsatisfactory and meagre evidence upon which this conclusion as to the cause of death is based in the majority of the cases. In some of the cases the gas found in the vessels was associated with the most ordinary advanced post mortem decomposition, which, as is well known, in some conditions may set in with great rapidity. Had there been no bacteriological examination in our case, the evidence in support of the view that air had entered the vessels during life would have been indeed stronger than in most of the reported cases. The patient had been weakened by copious haemorrhage, which in the writings on the subject is regarded as an important predisposing factor. A communication existed between the external air and an intrathoracic vessel. Death was sudden. The autopsy, made in cool weather eight hours after death without the slightest visible sign or odor of post mortem decomposition, revealed gas in large quantity in the vessels.

We have not been able to find any report of a bacteriological examination of the blood in the cases in which gas supposed to be air entering during life has been found in the vessels after death, and it is certainly surprising that no attempt has been made to determine by bacteriological examination whether or not what was taken to be atmospheric air might not be generated by the growth of the microorganism. The result of the examination in our case must necessarily cast doubt upon the interpretation hitherto accepted in many of the cases reported as death due to the entrance of air into the veins. A principal link in the chain of evidence has fallen out, viz., the belief that when the gas is found in the heart and vessels a few hours after death without any evidence of cadaveric decomposition, this is a proof that the gas is air, and is not the result of the development of microorganism. Hereafter in all similar cases a careful bacteriological examination, including anaerobic cultures, must be made before it can be admitted that the gas in the vessels has not been generated by microorganisms. Time only can determine whether such examinations will show that the explanation found to be correct in our case is exceptional or is applicable to many other cases which might otherwise be interpreted as due to the entrance of air into the vessels.

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[18] Fischer: Volkmann's Sammlung klin. Vorträge, No. 113.

[19] Greene: Am. Journ. Med. Sciences, 1864, Vol. 47, p. 38.

[20] May: Trans. Path. Soc. London, 1858, Vol. IX, p. 158.

OBSERVATIONS CONCERNING *BACILLUS AEROGENES CAPSULATUS*[1a]

(From the Pathological Laboratory of The Johns Hopkins University and Hospital)

In November, 1891, one of us (Welch[1]), at a meeting of The Johns Hopkins Hospital Medical Society, reported with demonstrations the discovery of a gas-forming bacillus found in the emphysematous tissues and blood of a man dead of aneurism of the aorta. In July-August, 1892, Welch and Nuttall[2] published a full report of this case and described the characters of the bacillus, for which they proposed the name *Bacillus aerogenes capsulatus.* Since this report there have been published, both in this country and in Europe, additional observations of the occurrence of this bacillus, and we have had the opportunity of studying a considerable number of cases in which this organism was found. We purpose in the present article, upon the basis of these unpublished reports and of our further observations, to consider the distribution and characters of this microorganism, and more particularly its relations to various affections and conditions of human beings.

We must refer to the original paper of Welch and Nuttall[2] for the full report of the case in which *Bacillus aerogenes capsulatus* was first found, for the detailed description of the morphological and cultural characters and the effects of intravenous inoculation of this bacillus, and for the deductions which were then drawn as to the probable relation of the bacillus to conditions characterized by the presence of gas in the blood and tissues. Here we shall give a summary of some of the salient points contained in this paper.

The patient was a mulatto man, aged thirty-eight, with tuberculosis and with aneurism of the ascending aorta which had perforated by a small opening through the anterior thoracic wall, and which had given rise to repeated external haemorrhages, the last occurring two days before death, which was sudden. No subcutaneous emphysema was noted during life. The autopsy was made in cool weather, eight hours after death, the body being still warm. There was no odor, greenish color, or other evidence of post mortem decomposition. There existed emphysema of the subcutaneous tissues of the

[1a] William H. Welch and Simon Flexner.

J. Exper. M., N. Y., 1896, I, 5-45.

greater part of the body. The blood vessels and heart contained abundant gas bubbles. The blood was laked, and the lining membrane of the heart and blood vessels showed diffuse imbibition of blood-coloring matter. There was gas in the blood-stained serum in the pericardial sac and in the peritoneum. Gas bubbles were present abundantly in the internal tissues, notably in the myocardium, the liver, spleen, and kidneys, these organs presenting the appearances described by German writers as those of *Schaumorgane*. Gas bubbles were numerous in the discolored, whitish, antemortem coagula which nearly filled the aneurismal sac. The gas burned with a pale bluish, almost colorless flame, a slight detonation being heard at the moment of ignition.

Upon microscopical examination of the organs there were observed around masses of bacilli frequently, but not always, disappearance of the nuclei and degenerative changes in the cardiac muscle cells and the epithelial cells of the liver and kidney, especially in the neighborhood of gas blebs, in the walls of which bacilli were often densely accumulated.

By examination of cover-slip preparations, a single species of bacillus was found in very large numbers in the blood and in all other situations in which gas was present. Aerobic cultures from the blood and organs remained sterile. Anaerobic cultures yielded numerous colonies of the same bacillus found in cover-glass preparations from the fresh blood and organs, no other species developing.

The morphological and cultural characters of the bacillus were described in full detail. Only the leading characters will here be recapitulated.

The bacillus is nonmotile, straight or sometimes slightly curved, variable in size, but averaging about the thickness of the anthrax bacillus and 3 to 6 μ in length, with adjacent ends slightly rounded or sometimes square cut; occurs singly, in pairs, in clumps, and sometimes in short chains, less frequently in threads and long chains; and stains readily with the ordinary aniline dyes, including Gram's and Weigert's fibrin stain, either uniformly or with small unstained spots, less frequently with isolated deeply staining granules. Capsules, although not constant, were frequently demonstrated, especially by Welch's method[3] for staining capsules, in specimens from the animal body and sometimes from agar cultures. No spores were found either in the animal body or in cultures.

The bacillus grows upon all ordinary culture media—rapidly at body temperature, slowly at temperatures as low as 18° to 20° C. It is anaerobic, no growth occurring on the surface of solid media or in ordinary fluid cultures in test tubes exposed to the air. There is growth in the depth of solid media exposed to the air. Under anaerobic conditions growth occurs both upon the surface and in the depth of solid media.

Gas is produced in all cultures containing fermentable material. In our plain nutrient agar or gelatin there is usually some formation of gas,[2a] and in media to which one per cent of glucose has been added there is at body temperature a rapid and very abundant development of gas.

The colonies in agar are grayish white to a more opaque white or brownish white color by transmitted light, sometimes with a central darker dot. At the end of twenty-four hours they do not exceed 0.5 to 1 millimeter, but they may subsequently attain a diameter of 2 to 3 millimeters, or even larger. Those in the depth are spheres or ovals, generally more or less flattened, usually, but not always, with irregular contours, the irregularity being due to knoblike or feathery projections. No putrescent or distinctive odor could be detected in the cultures, except sometimes a foul odor when masses of agar culture were crushed in a little bouillon.

In gelatin cultures there was observed a slow and limited liquefaction of the medium, manifested by a settling of the growth and by slight displacement of the gas bubbles in changing the position of the tube.

The bacillus produces at first diffuse cloudiness of sugar bouillon; later the fluid becomes clearer, with an abundant whitish, more or less viscid sediment. In twenty-four to forty-eight hours milk is coagulated, the clot being generally firm, retracted, and furrowed with the marks of gas bubbles. In anaerobic potato cultures there is either no visible growth or a thin, moist, grayish white growth on the surface and abundant formation of gas in the fluid accumulated about the bottom and sides of the potato. In ascitic fluid there is abundant growth with formation of gas bubbles.

The vitality of the cultures is of very variable duration, according to the character of the medium and the mode of cultivation. Cultures in sugar bouillon in Liborius tubes filled with hydrogen were found to be dead in three days or less. On the other hand, sugar-bouillon cultures in Buchner's[4] jar may survive four months and longer. Cultures in sugar media are shorter lived than those in plain media. Cultures in tubes hermetically sealed after two or three days' growth survived several months. The thermal death point, determined by Sternberg's[5] method, was found to be 58° C. for ten minutes.

[2a] As has been pointed out by Theobald Smith, the amount of sugar in ordinary meat bouillon varies with the character of the meat and the length of time it has been kept. In some meat and meat kept a considerable time there may be no sugar. The gas produced by fermentation of carbohydrate, therefore, varies in amount in different lots of media made with plain bouillon, and may be absent altogether. These facts will explain many of the discrepancies of statement of different observers as to the production of acid and of gas by various bacterial species in nutrient media to which sugar has not been added.

Experiments upon animals were made chiefly to elucidate the relation of the bacillus to the conditions found at the autopsy upon the patient. With one important exception, to be mentioned subsequently, it was determined that the bacillus is not pathogenic for rabbits when introduced directly into the circulation.

If the animal be killed immediately or soon after the intravenous injection of 0.5 to 1 cubic centimeter of bouillon culture, after eighteen to twenty-four hours at the temperature of 18° to 20° C., and after four to six hours at temperatures from 30° to 35° C., the bacilli are found abundantly, together with great formation of gas, in the blood vessels and organs. The viscera present the typical characters of *Schaumorgane.* All of the conditions relating to the bacilli and their production of gas which existed at the post mortem examination of the patient were reproduced experimentally in animals.

When the bacilli are introduced at one place in the vascular system soon after death, they develop in the course of the vessels into which they have been introduced, and the time required for them to appear with gas formation throughout the vessels is much longer than when they have been distributed throughout the circulation just before death.

These experiments upon animals were considered to favor the view that in the patient the bacilli had entered the circulation before death, and probably primarily from without through the opening in the thoracic wall into the aneurismal sac. The multiplication of the bacilli in the vessels and the formation of gas were regarded as occurring mainly post mortem.

The bearing of these observations upon various conditions of the body characterized by the presence of gas was considered, particularly upon the reported cases of death from the entrance of air into the circulation and upon cases in which gas, unassociated with ordinary cadaveric decomposition, was found in the blood vessels after death without the existence of any opening through which air could enter the circulation.

In view of the positive result of an intravenous inoculation of a pregnant rabbit with a culture of *Bacillus aerogenes capsulatus,* especial emphasis was laid upon the possible relationship of this bacillus to the reported deaths attributed to the entrance of air into the uterine veins in the puerperal state, after abortions, and after injections into uterine cavity. As the explanation of these cases suggested by Welch and Nuttall[2] has since been confirmed, we will introduce here the protocol of the original experiment upon the rabbit.

Experiment 9.—Pregnant rabbit, 1 cubic centimeter of sugar bouillon culture (ninth generation), forty-eight hours old, into ear vein. Rabbit was found dead twenty-one hours after the inoculation, the body being still warm, although stiff. The death was sudden. The rabbit was alive half an hour previously, and apparently in good condition. The autopsy was made six hours after death. By this time the body, which has been in a room at a temperature of about 18° C., is much swollen, the abdomen distended, and emphysematous crackling can be felt over the abdomen and the lower part of the thorax. Bloody fluid containing gas bubbles oozes from the vagina. On opening the peritoneal cavity gas escapes freely, which burns with a colorless flame, a slight explosive sound being heard upon first applying the match. This cavity contains blood-stained fluid. Blood vessels, both veins and arteries, contain gas. The heart cavities on both sides contain soft dark clots with gas bubbles. Spleen is somewhat enlarged, soft, and dark red. Liver does not contain gas blebs, but gas is present in the hepatic vessels. The abdominal vena cava is blown up with gas. The uterus contains six embryos about three centimeters long. The sacs of the uterus corresponding to these embryos are greatly distended with gas. Two of the sacs are more distended than the rest, their walls are very thin, contain gas bubbles, and look as if they were just ready to burst. The embryos in these two sacs are macerated. dark livid red, partly destroyed and smaller than the others, which are intact. The placentae contain gas bubbles in large amount.

Cover-slip preparations show an enormous number of characteristic capsulated bacilli in the distended uterine sacs, and a large but not so great number in the liver, spleen, heart, and blood vessels. Cultures from the uterus, spleen, and left ventricle are pure of the typical gas-forming bacillus which was used for the injection.

It seems probable from the autopsy that two of the embryos in the uterus were already dead when the injection was made, and that in these embryos and the part of the uterus containing them the bacilli were able to gain a foothold and develop. The extent of development of the bacillus and of gas was much greater than is observed at so short a period after death (six hours) when the animal is killed immediately or shortly after intravenous injection of the cultures and is kept at the temperature of the room.

Inasmuch as we now know, from observations made since Welch and Nuttall's publication,[2] that *Bacillus aerogenes capsulatus* is a widely distributed organism, of not infrequent occurrence in the human body, and that it may be associated with various pathological conditions, we have searched through the literature in order to find whether it may not have been recognized by earlier observers. It seems highly probable that it must have been seen before its isolation and description by Welch and Nuttall.[2]

It is not a little remarkable that of the considerable number of reported cases of suspected entrance of air into the blood vessels, none seem to have been previously the subject of bacteriological study. For some of the cases in which gas was found at the fresh autopsy widely distributed in the blood vessels and tissues several writers have recognized the difficulties involved in the usual explanation that the gas was atmospheric air or the result of ordinary cadaveric putrefaction. When this condition was found associated at autopsy with an emphysematous phlegmon or gangrene, some observers have recognized the specific bacterium causing the latter affection during life as also the cause of the development of gas in the blood and internal organs either during life or after death.

In the various reports of cases of gas in cysts, joints, serous cavities, urinary passages, and uterus we have been unable to find at a date prior to that of Welch and Nuttall's publication[2] any description of a bacterium resembling *Bacillus aerogenes capsulatus.*

On the other hand, we have little or no doubt that in the cases reported before 1892 under the names malignant oedema, emphysematous or gaseous gangrene, gaseous phlegmon, emphysematous cellulitis, gangrenous septicaemia, gangrène gazeuse foudroyante, etc., the bacilli usually described as those of malignant oedema were in some instances identical with *Bacillus aerogenes capsulatus.* We shall consider subsequently in this article the possible relationship of these bacterial species, but in this connection it may be said that when in the class of cases under consideration the identification of an anaerobic bacillus, as that of malignant oedema, is based solely upon general morphological resemblances and similar pathogenic effects, without determination of motility and development of spores, the bacillus in question may, in the light of our present knowledge, equally well be regarded as *Bacillus aerogenes capsulatus.*

There are not a few cases which fall under this latter category, but in these the characters of the bacilli have not been described with such detail as to permit their positive identification. On this account it does not seem to us worth while in this article to attempt to disentangle as to this matter the generally unsatisfactory and incomplete descriptions of supposed malignant oedema bacilli in this group of diseases in human beings.

The case reported in 1888 by Bremer[6] as one of malignant oedema may serve as an illustration of the cases which we should regard as probably referable to *Bacillus aerogenes capsulatus.* In this case, at an autopsy made seventeen hours after death of a woman who developed as a sequel to an abortion emphysematous swelling of the right pectoral region and adjacent arm, Bremer found most extensive development of gas in the blood vessels, the tissues, and the thoracic and abdominal viscera, with an enormous number of bacilli, devoid of spores, in the uterus, blood, organs, and serous cavities. The bacilli were said to resemble *Bacillus oedematis maligni.* Nothing is said as to motility or threadlike forms of the bacillus, nor was it obtained in culture. The inoculation of guinea pigs with serosanguinolent fluid containing the bacilli was fatal in twenty-four hours, with lesions said to be like those of malignant oedema.

Of cases of gaseous phlegmon reported before 1892 in which anaerobic bacilli somewhat resembling the bacillus of malignant oedema, but not identified with it, were found, those of F. J. Rosenbach,[7] Wicklein,[8] and Levy[9] have received special consideration by E. Fraenkel[10] in his monograph *Ueber Gasphlegmonen.* He comes to the conclusion that only the bacillus described by Levy[9] in 1891 is probably identical with *Bacillus aerogenes capsulatus.*

Rosenbach's bacillus formed spores and he was unable to cultivate it. *Bacillus emphysematis maligni* of Wicklein was motile, grew in long, jointed threads, formed spores both in cultures and in the animal body, and was pathogenic only for guinea pigs. In spite of the contrary conclusion reached by Wicklein, Fraenkel is inclined to identify this bacillus with that of malignant oedema.

The bacillus isolated by Levy[9] in 1891 from a gaseous phlegmon of the pelvis and thigh of a puerperal woman was anaerobic, gas-producing, non-motile, short, small, delicate, stained by Gram, grew in long threads and chains of thirty and more members, and formed anthraxlike colonies. It grew only at body temperature, and was of so short viability that it was cultivated only in the first generation. No experiments upon animals were made. The rapid healing of the abscess was attributed by Levy to the short viability of the bacillus. In a subsequent article published in 1895 Levy[11] declares this bacillus to be identical with Fraenkel's *Bacillus phlegmones emphysematosae,*[12] but we do not consider that Levy's original description of this bacillus suffices for such identification, especially in the absence of experiments on animals; nor can his description of the bacillus as short, small, or delicate (*"kurze, kleine, feine Bacillen"*), and as incapable of growth below the body temperatures, be reconciled with the characters of *Bacillus aerogenes capsulatus.*

As stated by Welch and Nuttall,[2] the bacillus described by E. Fraenkel,[13] in 1899, in a case of gastritis emphysematosa may be identical with our bacillus, but it was studied only on hardened specimens of the stomach.

It would not be surprising if *Bacillus aerogenes capsulatus* should be found among the considerable number of anaerobic gas-producing bacilli which have been isolated from the soil, the faeces, decomposing infusions of animal or vegetable matter, and other sources. Most of these bacilli are motile and sporogenic. Many differ from each other only in such minor characters that their separation into distinct species is difficult or impossible. As there is reason to believe that *Bacillus aerogenes capsulatus* may exist in the soil, we have examined especially the descriptions of the various bacilli isolated from this source by Liborius,[14] Lüderitz,[15] Sanfelice,[16] and others. But we have not found a bacillus described which we are able to identify in all respects with our bacillus, although we can by no means exclude in every case the possibility of identity. The same statement applies to the several varieties of malignant oedema, pseudooedema, and Rauschbrand bacilli which have been described. There is usually some point regarding motility or spore formation or conditions of growth or odor or other cultural character or the result of or absence of animal experiment which prevented such identification. We shall have something to say subsequently as to the value of such points as these in the differentiation of species among this group of anaerobic bacilli.

The first observations of *Bacillus aerogenes capsulatus* following the report of Welch and Nuttall[2] were made by E. Fraenkel, who published, in January, 1893, a preliminary report,[12] and later in the same year a monograph concerning gaseous phlegmons.[10] Fraenkel makes no mention of the previous discovery of the same organism by Welch and Nuttall,[2] and doubtless was unacquainted with their work. To Fraenkel[10] belongs the credit of demonstrating the causal relation of *Bacillus aerogenes capsulatus* to the affection called variously gaseous phlegmon, emphysematous cellulitis or gangrene, gangrène gazeuse, etc.

Fraenkel[10–12] in these communications reported the results of his examination of four cases of gaseous phlegmon, in one of which the gas bacillus was found unmixed with other bacteria, whereas in the remaining three it was mixed with other bacteria, chiefly pyogenic cocci. In the case of pure infection with the gas bacillus there was no suppuration in any part of the enormous emphysematous swelling of the right lower extremity. On incision a turbid brownish fluid, without offensive odor and containing gas bubbles, escaped. The muscles were completely disintegrated into an opaque reddish gray pulp filled with bubbles of gas. It is noteworthy that in two of the cases the gaseous phlegmon followed hypodermic injections, in one case of camphor oil and ether and in the other of a dilute solution in water of sulphuric acid and chloride of morphine. The latter was the case of unmixed infection and pursued a rapidly fatal course, death occurring two days after the injection. Three of the cases terminated fatally; in one the issue is not stated. In none of the cases was gas found at autopsy in the internal organs or blood vessels.

The bacillus isolated by Fraenkel[10] from these cases corresponded in all essential morphological and cultural characters with *Bacillus aerogenes capsulatus*. To it Fraenkel gave the name of *Bacillus phlegmones emphysematosae*. As regards the formation of spores, he states that this is inconstant and exceptional, and was observed by him only in a few of the bacilli in cultures in one lot of agar containing formate of sodium. Spores were not noticed in other agar cultures, even when the medium was made according to the same formula with formate of sodium. A clear zone was noticed around bacilli from the animal body and from surface agar cultures, but no capsule was stained. The probable identity of Fraenkel's bacillus of gaseous phlegmon with *Bacillus aerogenes capsulatus* was recognized by Mann,[17] in an article published in February, 1894, who worked in our laboratory, and by one of us (Welch)[18] in a published article on "The General Bacteriology of Surgical Infections." Through the kindness of Dr. Fraenkel we have been enabled to examine cultures of his bacillus which he has sent us, and we have found this organism in every respect identical with that described by Welch and Nuttall.[2] This identity is admitted in a recent communication by Goebel,[19] whose observations come from the same hospital in Hamburg with those of Fraenkel, and in a personal communication from Dr. Fraenkel to one of us (Welch) the same opinion is expressed.

As the experiments of Welch and Nuttall[2] were conducted to throw light upon the relationship of the gas bacillus to conditions found in their patient, so those of Fraenkel were for the purpose of determining its relation to gaseous phlegmons. Conducted thus from different points of view, these two sets of experiments are mutually complemental, and taken together they have established the main facts pertaining to the effects of inoculation of animals with this microorganism. Fraenkel's experiments were made by inoculation of mice, rabbits, and guinea pigs. No result followed subcutaneous injection of cultures into mice. Rabbits survived intravenous inoculation without any apparent disturbance. Sometimes, but not constantly, the subcutaneous inoculation of rabbits was followed by local oedema with necrosis of the overlying skin, from which the animal recovered. The presence of gas in the swelling is not mentioned.

In guinea pigs, however, subcutaneous inoculation of cultures of the bacillus caused a process essentially identical with the condition observed in the human patients. The first effect was a painful infiltration of the tissues with a blood-stained fluid extending from the site of inoculation. In more than half of the cases gas appeared in the swelling within the first twenty-four hours, in the remainder at a later period. Death may occur in twenty-nine to thirty-six hours after inoculation or at a later period. In a considerable number of cases the skin bursts over the infiltrated area, and then after prolonged necrosis and ulceration recovery by cicatrization may occur. There was no actual suppuration unless pyogenic bacteria were mixed with the gas bacillus. At autopsy the muscles of the affected area were converted into a soft, pulpy mass and blood-stained fluid infiltrated the part, which contained gas bubbles in large number. There may be direct extension of the inflammation to the peritoneal and pleural membranes. The bacilli were found only in the actually inflamed and necrotic parts, but here in enormous number, occurring in the form both of rods and of threads.

The next publications concerning *Bacillus aerogenes capsulatus* were those of Graham, Steward and Baldwin,[20] and of P. Ernst,[21] both of these articles appearing in August, 1893. The case reported by Graham, Steward and Baldwin is of extraordinary interest. The patient was a laboring woman, married, aged thirty-five. About fourteen hours before death the woman, who had previously been well, was seized with what she described as a severe chill, which lasted more than four hours. This was followed by pain in the region of the uterus and ovaries and by vomiting and purging.

She was first seen by Dr. Steward at 1 p. m., about five hours after the onset of the first symptoms. She was then extremely restless and suffering from " distress and pain " in the lower abdominal region. There was a slight flow of bright red blood from the uterus. Abortion was suspected. The pulse was about 120. Morphine was administered hypodermically. The patient when seen at 3.30 p. m. appeared much relieved and dozed off to sleep. She was not visited again by Dr. Steward until half past nine in the evening, when he found her "emphysematous from the top of her head to the soles of the feet." Emphysematous crackling could be felt over the entire surface of the body. There was a diffuse blood red color of the whole surface of the body. The skin was smooth. The husband said that the swelling, redness, and crackling began about 5.30 p. m. The pulse was weak; the breathing somewhat rapid. We quote from Dr. Steward's report: " The patient said that she was not now suffering so much pain, but that she felt bloated, with a sense of numbness over the entire surface of the body. She had not passed urine since morning. I introduced a catheter and drew only a few drops of bloody urine. Noticing that her breathing was becoming very short and quick, I felt for the pulse. It was gone. The heart had stopped beating. The woman was dead. There was no death struggle. The patient was entirely conscious and talking to within three minutes of death. There was no change in the ruby appearance of the skin at the approach or instant of death."

The emphysematous condition increased so rapidly after death as to alarm the undertaker. Eighteen hours after death the body was bloated to more than double its natural size. The autopsy was made about twenty hours after death. The uterus gave evidences of a recent abortion at about the third month. It contained bloody fluid and placental *débris,* and its interior presented suspicious marks, but no positive evidence of criminality was obtained. The vessels, tissues, and organs were enormously distended with gas, which ignited with an explosive sound, and was determined by chemical examination to be largely hydrogen. The bacteriological examination made by

Graham showed in the blood in very large number and pure culture bacilli, which were identified by microscopical examination, cultures, and the inoculation of animals as *Bacillus aerogenes capsulatus*. Dr. Graham kindly sent one of us (Welch) microscopical specimens and photographs of the bacillus, which in the characters thus revealed we found to be indistinguishable from our bacillus.

This case was recognized by Baldwin and Graham before the autopsy as probably referable to the bacillus previously described by Welch and Nuttall, and reference is made by Baldwin to the brief report of a case presenting similar post mortem appearances in the same journal of October, 1892.

The significance of the remarkable case reported by Graham, Steward and Baldwin[20] is that it affords positive evidence that the gas bacillus may invade the blood and produce gas widely distributed over the body during life. The development of extensive emphysema of the entire subcutaneous tissue of the body in this case during life and within the space of four hours cannot well be explained otherwise than upon this supposition. The portal of entry of the bacillus was manifestly the uterus, which presented unmistakable evidences of a recent abortion, possibly artificially produced by the insertion of some instrument. This case therefore confirms the explanation suggested by Welch and Nuttall of deaths previously reported as due to the entrance of air into the uterine veins after abortions.

The attention of P. Ernst was arrested especially by the formation of foam upon the surface of the liver at autopsy, this foam being due to the development of gas by *Bacillus aerogenes capsulatus*. He reported in August, 1893, two observations of this foaming liver (*Schaumleber*).[21] These cases were observed prior to the publication of Welch and Nuttall,[2] which led Ernst not to delay longer the report of his own studies upon the same subject.

The first case was that of a woman who developed putrid endometritis and septic peritonitis after removal of a macerated four months' foetus, the uterus, through doubt as to the diagnosis, having been previously curetted, sounded, and tamponed. No development of gas in the tissues was noted during life. At the autopsy, made only three hours after death, the entire uterine wall crepitated from the contained gas. Coagula in the right iliac vein and conus pulmonalis contained gas. Gas was found also in the myocardium, and especially in the liver, from the cut surface of which gas oozed up and in a few hours formed a layer of foam several centimeters thick.

A similar condition of the liver was observed at an autopsy made eighteen hours after death upon a case of peritonitis resulting from traumatic perforation of intestine in a hernial sac. The patient died four hours after a herniolaparotomy. Green discoloration of the liver and other parts indicated advanced cadaveric decomposition. Gas was found in the liver and gas blebs in the intestinal mucosa.

The organism found in these two cases was a nonmotile, anaerobic bacillus corresponding in its essential features with *Bacillus aerogenes capsulatus,* with which it was identified by Ernst. Rods exceeding 5 μ in length were met only very exceptionally. Capsules were not stained and growth in gelatin at room temperature was not obtained, but it is not clear how thoroughly examinations were made as to these points. Only a few experiments upon animals were made. It is noteworthy, in view of the negative results of Fraenkel, that Ernst succeeded in killing two mice by subcutaneous inoculation with the bloody fluid from the livers, the characteristic bacilli being found, at least in one of the mice, in the heart's blood, liver, and spleen. Subcutaneous inoculation of a guinea pig with hepatic fluid from the first case caused death in less than twenty-four hours, with foul smelling gangrene of the skin and sanguinolent oedema, which contained a variety of cocci and bacilli besides the typical gas bacillus. The guinea pig inoculated with material from the second case survived. A rabbit inoculated into the ear vein with blood rich in bacilli from the first case became manifestly ill and voided turbid bloody urine containing the characteristic bacillus and long threads, which were regarded as possibly belonging to the same species. Contrary to expectation, the rabbit recovered.

Ernst[21] describes with much detail the histological changes in the liver, these being characterized, as pointed out by Welch and Nuttall,[2] by disappearance of nuclei and degenerative changes around masses of bacilli. The characters of these alterations are believed by Ernst to indicate the lodgment of the bacilli in the liver during life. The development of gas holes in the liver, however, he regards as post mortem.

In February, 1894, Mann[17] reported a case of emphysematous gangrene of the hand due to *Bacillus aerogenes capsulatus* associated with *Streptococcus pyogenes.* The patient was in Dr. Tiffany's clinic at the University of Maryland. The affection began as a small black blister on the palmar surface and near the end of the left ring finger. The entire hand became swollen and oedematous, and upon palpation emphysematous crackling could be felt up to the wrist. The temperature rose to 105.5° F. and the pulse to 130. Free incisions were made and the hand placed in a hot sublimate bath. Recovery, with loss of the gangrenous extremity of the middle finger, took place.

Aerobic cultures were pure of *Streptococcus pyogenes.* By means of anaerobic plate cultures *Bacillus aerogenes capsulatus* was isolated in purity and was fully identified by Mann, who kindly submitted to me his cultures.

Mann calls attention to the similarity of the lesions produced by *Bacillus oedematis maligni* and *Bacillus aerogenes capsulatus,* which he regards as distinct species.

In May, 1895, Levy,[11] under the title of pneumothorax without perforation, published an observation of *Bacillus aerogenes capsulatus.* In a patient forty-eight years old, after repeated aspirations of clear fluid from the left pleural cavity, there developed pneumothorax. The slightly turbid serous fluid withdrawn by aseptic aspiration eight days after the first appearance of the signs of pneumothorax, as well as the opaque fluid removed a week later by resection of a rib, furnished in anaerobic cultures a short, thick, plump, nonmotile, gas-producing bacillus, devoid of spores, staining by Gram, occurring usually as a diplobacillus, but not infrequently also as jointed threads, and growing both at body and room temperatures. Bouillon cultures possessed a peculiar viscid consistence, such as is customary with capsulated bacteria, but no distinct capsule could be demonstrated. Subcutaneous or intraperitoneal inoculation of guinea pigs caused reddish serous exudate filled with gas bubbles at the site of inoculation. In one guinea pig a few bacilli were found in the blood as well as in the exudate.

Levy [11] interprets the case as primarily one of tuberculous pleurisy to which in some unknown way the gas bacillus gained access and caused during life gas formation without perforation. At the autopsy by von Recklinghausen a fibrous mass the size of an egg containing caseous foci was found in the upper part of the right lung, and a small caseous focus was found in the lower lobe of the left lung without rupture of the pleura. The possibility of a healed perforation of the pleura could not be positively excluded, but this was considered by von Recklinghausen to be improbable. Unfortunately, nothing is said as to the results of aerobic cultures, nor whether other bacilli than the one described were present, nor as to the direct microscopical examination for bacteria of the pleuritic exudate.

Levy identifies the bacillus isolated from this case with that obtained from the case of rapidly healing gas abscess already cited, although the bacillus from the latter case was described as small and delicate and incapable of growth at ordinary temperatures and was only imperfectly studied. He doubtless correctly identifies the bacillus from the case of pneumopleuritis with Fraenkel's bacillus of gaseous phlegmon. With the exception of his own first doubtful observation of this bacillus and of Fraenkel's studies, he makes no mention of any of the other observations of the same bacillus.

In July, 1895, Goebel[19] published an article[3a] concerning the bacillus of *Schaumorgane,* confirming the results of Welch and Nuttall, and demonstrating that this bacillus is identical with *Bacillus phlegmones emphysematosae* of Fraenkel. He cultivated the bacillus from three cases at autopsy. The first was a case of pyelonephritis consecutive to papilloma of the bladder and abscesses of the prostate and seminal vesicles. Gas blebs were found in the heart muscle, liver, spleen, adrenals, and stomach. Around many, but not all, of the blebs in the heart and liver the cells were necrotic. Necroses were not present in the spleen around the gas holes.

The second case was one of enlarged prostate which had been catheterized. There existed general arteriosclerosis, pulmonary emphysema, lobular pneumonia, and embolism of branches of the pulmonary artery. Numerous gas blebs were found at autopsy beneath the mucous membrane of the urinary bladder. Necroses were absent from the walls of these blebs.

The third case was one of the pyaemia following endophlebitis of the cutaneous veins of the left foot. Beneath the gastric and intestinal mucosa were gas blebs resembling those in the preceding case, except that necroses were present in the neighborhood of the blebs.

The gas vesicles in all of these cases contained numerous bacilli in their walls and interior. The characters of the bacillus isolated from these cases correspond in all particulars with *Bacillus aerogenes capsulatus* of Welch and Nuttall[2] and *Bacillus phlegmones emphysematosae* of Fraenkel. Capsules were stained by Goebel[19] around bacilli from the animal body, but apparently only once around bacilli from cultures. The experiments of Welch and Nuttall were repeated with identical results both with the bacillus isolated from foaming organs and with that from gaseous phlegmons. Necroses were absent from the walls of gaseous blebs which formed in the organs of rabbits which had been killed a few minutes after intravenous injection of cultures. Spore formation was observed only in one culture, this being a forty-eight-hour stab culture in sodium formate agar. Liquefaction of gelatin with cultures from the first case was slow and limited; with those from the second it was rapid and marked. Subcutaneous inoculation of guinea pigs produced the same effects as those described by Fraenkel in his monograph on gas phlegmons.

[3a] This article of Goebel is described as an extract from an elaborate paper which is to appear in the Jahrbücher der Hamburgischen Staatskrankenanstalten. In the latter Goebel[22] promises a detailed consideration of the differential diagnosis of *Bacillus aerogenes capsulatus* from other similar anaerobes (malignant oedema, Rauschbrand, etc.), and further conclusions to be drawn from his observations. This volume of the Jahrbücher has not yet appeared (December, 1895), so far as we have been able to learn.

In the first and third cases it was considered, in view of the presence of necroses, that the gas bacilli invaded during life the organs containing the blebs at autopsy, whereas, in the second case, the invasion of the bacillus is regarded as post mortem.

Passow[23] has recently reported a case of gaseous phlegmon of the right shoulder joint which was observed in November, 1893. Three weeks after a superficial wound of the hand which healed slowly by suppuration the patient developed septicaemia with purulent panophthalmitis, and a few days later an emphysematous swelling around the right shoulder joint and extending down the arm. Upon incision, stinking, brownish, nonpurulent fluid containing gas with the odor of sulphuretted hydrogen was evacuated. Three days later the patient died. Gas was not found at the autopsy in the blood or internal organs. The bacillus isolated from the gaseous phlegmon was imperfectly studied, but is identified by Passow with Fraenkel's bacillus of gaseous phlegmon. It caused rapid liquefaction of gelatin which gave a penetrating odor of sulphuretted hydrogen. Clear spaces were seen in the stained bacilli, but it was not determined whether these were spores. Cultures and the wound secretion were not pathogenic for mice and rabbits. Passow's description of his bacillus hardly suffices for its identification, and the strong odor of sulphuretted hydrogen in gelatin cultures is not in favor of its being *Bacillus aerogenes capsulatus,* if the culture was pure. It is not even clear from Passow's statements that the bacillus was incapable of aerobic growth, or that it was separated in culture from the staphylococci which were also present in the phlegmon.

The preceding observations are all which we have found relating to *Bacillus aerogenes capsulatus* in the literature since the publication of Welch and Nuttall.[2] There have been reports of cases by Pantzer,[24] Ross, and others of gas in the tissues and serous cavities, in which the opinion has been expressed that this bacillus was concerned, but bacteriological examinations were not made. Reference will be made subsequently to reports of cases of gaseous phlegmon, gas cysts, etc., in which other bacteria than our bacillus were found.

We will now report our personal observations of additional, hitherto unpublished, cases in which *Bacillus aerogenes capsulatus* was found either during life or at autopsy. Only such details of these cases will be given as are necessary for an understanding of the relations of the bacillus to the case.

The methods of bacteriological examination adopted have been essentially those described by Welch and Nuttall,[2] and their description need not be repeated in connection with the report of each case. In all cases cover-glass preparations were examined from the original material. Anaerobic cultures were made usually in one-per-cent glucose agar. Occasionally sodium formate agar was used. We employed, as a rule, the Buchner method,[4] less frequently displacement of the oxygen by hydrogen. Pure cultures were obtained from single colonies, and were planted in various media.

We made continual use of the intravenous inoculation of rabbits, which were killed by a blow at the back of the neck a few minutes after the inoculation. At suitable temperatures the development of the bacilli and of gas in these dead rabbits is rapid and extensive. In this procedure the body of the dead rabbit previously inoculated in the manner described may be regarded as a receptacle containing the best possible nutritive medium and presenting the best conditions for anaerobic growth so far as this bacillus is concerned. By making cultures from these rabbits at a period not too long after the inoculation it was often possible to separate the bacillus from other microorganisms which might be mixed with it.

Rabbits, guinea pigs, pigeons, and mice were inoculated subcutaneously and pathogenic effects noted. The results of the experiments upon these animals will be described after consideration of the cases in which the gas bacillus was found. We shall also reserve to a later consideration certain variations observed in the characters of the bacillus and its differentiation from allied anaerobic bacteria.

Unless otherwise stated, the cases were observed in The Johns Hopkins Hospital and Pathological Laboratory. They are arranged as far as possible in groups, but not in the chronological order of their occurrence. On account of their number they are presented in as abbreviated a form as is consistent with clearness. Our autopsies are, as a rule, unusually fresh, and the bodies are kept after death in a large ice chamber. They were made by one of us (Flexner), also by Drs. Barker and Blumer for whose assistance in our studies we desire to express our thanks.

I. Boy, thirteen years old, admitted to the service of Dr. Halsted, March 29th, fourteen hours after gunshot wound of left knee. Examination upon admission showed an ecchymosed bullet wound at the lower outer edge of the patella, with effusion into the knee joint. Flexion and extension gave rise to a peculiar sound, resembling the passage of gas along the intestine. The diagnosis of gas in the joint was made. No emphysema of extraarticular tissues. Temperature, 101.5° F. Twenty-three hours after the injury the joint was freely opened by Dr. Halsted. It contained partly coagulated blood and bloody fluid in which many gas bubbles were present. Tissues about the joint oedematous. A large conical bullet was chiselled from the head of the tibia a short distance below the cartilage. The operation was aseptic throughout, only sterilized instruments coming into contact with the bullet. After the operation the joint was freely irrigated with sublimate solution (1 to 1,000). On account of the persistence of high temperature and the extensive infection of the tissues above and below the joint, the thigh was amputated at the upper third on April 5th. The wound was treated by the open method in the bath, and there was ultimate recovery. Examination of the amputated leg showed extensive soft, reddish yellow necrosis of the muscles, with separation of the muscles by bloody oedema and sloughy tissue. Gas bubbles were present in various parts of the affected tissues in moderate number. There were some foci of pus, but, in general, the process was one of necrosis with oedema rather than of suppuration.

Cover-slips from the blood in the joint at the first operation showed numerous bacilli with the morphology of the gas bacillus and a smaller number of cocci. Anaerobic and aerobic agar cultures were made both from this bloody fluid and from the bullet, which, seized by sterile forceps, was immediately dropped into a tube of high melted agar. Numerous colonies of *Bacillus aerogenes capsulatus* developed in the anaerobic cultures, most rapidly and abundantly from the one inoculated with the bullet. In smaller number there grew colonies of *Staphylococcus aureus* and *Streptococcus pyogenes.* Cultures and cover-slips from the second operation still showed the gas bacillus, but now the streptococci predominated. Typical gas development in blood and organs followed intravenous inoculation of rabbits which were killed soon after the inoculation. The bacillus caused the death of pigeons in seven to twenty-four hours, with the characteristic lesions. Gaseous phlegmon followed subcutaneous inoculation of guinea pigs.

The notable features of this case are the infection of the point by a bullet carrying the gas bacillus, the rapid development of gas in the joint, so that its presence could be recognized by manipulation fourteen hours after injury and before the joint was opened by the surgeon, the rapid extension of the necrotic and gaseous inflammation to the surrounding tissues, and the ultimate recovery after amputation.

II. November 9th, man admitted to the service of Dr. Halsted with the history that his left leg had been run over by a car and shortly before admission had been amputated at about the junction of the middle and lower third of the leg. On removing the dressings, which were saturated with blood, there was so much bleeding from the stump that an Esmarch bandage was applied above the knee. The skin was covered with dirt and sand, and showed no evidence of having been cleansed before the amputation. The wound was opened and irrigated, and after ligation of the vessels it was partly closed and drained with gauze. At this time there was no emphysema of the tissues. Twenty-four hours later the temperature rose with a chill to 104° F., the pulse ranged from 120 to 160, and there was much pain and tenderness in the leg. Extending from the edges of the wound nearly to the patella, the skin, which was not much reddened, was the seat of emphysematous crackling over the anterior and lateral aspects of the leg. Free incisions were made and gas was found in the subcutaneous and intramuscular tissues with extravasated blood and bloody turbid exudate. Although temporarily relieved, the condition of the patient became critical, and amputation was done at the lower third of the thigh by Dr. Bloodgood. After this the patient made a good recovery.

Cover-slips from the blood-stained fluid, which escaped at the first incision into the emphysematous tissues, contained numerous bacilli with the morphology of the gas bacillus, many being contained within leucocytes with polymorphous nuclei, and many also being free. Chains and clumps of cocci were also seen. Cultures gave *Bacillus aerogenes capsulatus* with typical reactions and, in addition, *Staphylococcus albus.*

This case belongs also to the group of gaseous phlegmons, infection with the gas bacillus being probably referable to the dirt which was ground into the leg by the passage over it of a car wheel. As in the preceding case, the gas bacillus was associated with pyogenic cocci. Here also recovery followed amputation, the previous treatment by free incisions not having held the infectious process in check.

III. June 2d, man admitted to the service of Dr. Halsted with the history that eight days previously he had fallen from a wagon and injured his right hip and thigh. On admission, temperature 101° F., subsequently rising to 104°; pulse, 80 to 90. Thigh flexed on the pelvis, the leg on the thigh, the limb being abducted and rotated outward. No external wound of integument. The thigh, especially on the inner and posterior aspects, much swollen and tender, with emphysematous crackling on the inner side and in the popliteal space. The skin over these parts is reddened. A similar condition in the gluteal region. By rectal examination a tender swelling can be felt about four centimetres above the anus. An incision was made from the tuberosity of the ischium to the popliteal space. The subcutaneous tissues were infiltrated with serum. The deep fascia was tense and thickened by a fibrinous exudate. Upon incising this tense fascia a turbid grayish fluid mixed with gas bubbles and having a faecal odor escaped. The muscles and tissues were much disintegrated. As the process extended down the leg, incisions were continued to the heel, the whole lower extremity being red, swollen, and emphysematous.

Death on June 7th. Autopsy twelve hours after death. The tissues of the gluteal region and right lower extremity extensively necrotic and infiltrated with offensive, dirty, turbid, grayish red exudate. The muscles are friable and distintegrated. In places there is pus. Gas bubbles are present, most abundantly in the gluteal region. The necrotic inflammation which has dissected out the sciatic nerve can be followed through the sciatic notch into the pelvis, where there is a perirectal phlegmon the size of an orange pushing up the peritoneum, which is greenish in color over the prominence but not inflamed. The perirectal abscess communicates by two perforations with the rectum. Moderately large probes can be passed from the rectum through these openings. Peritoneum smooth, without excess of fluid. No gas in the heart, vessels, or viscera. No fracture of the pelvis.

Cover slips from the gas-containing fluid which escaped at the incision into the thigh during life showed a large number of bacilli with the morphology of the gas bacillus. This was the predominant organism, but in addition were seen a short, oval bacillus, slender bacilli, and cocci in long and short chains, the picture being suggestive of that of intestinal bacteria. The cultures gave abundant colonies of *Bacillus aerogenes capsulatus,* which was tested upon animals, and also streptococci. The same bacteria were found by cover slips and cultures at autopsy in the necrotic inflamed tissues. Aerobic and anaerobic cultures from the blood and organs were sterile except that the gas bacillus was isolated in anaerobic culture from the congested lower lobes of the lungs, where it was also demonstrated by direct microscopical examination.

In this case a most extensive fatal emphysematous cellulitis followed traumatic rupture of the rectum, the process extending rapidly from the ischiorectal tissues through the sciatic notch into the gluteal region and down the thigh, nearly the whole lower extremity becoming involved. It is clear that *Bacillus aerogenes capsulatus* in this case escaped with other bacteria from the intestine through the rectal perforations, and grew during life abundantly in the tissues.

IV. For the following unpublished case we are indebted to Dr. Tiffany, Professor of Surgery in the University of Maryland, who has kindly furnished us the history. A vigorous telegraph lineman, twenty-seven years old, at 10.30 a. m. fell from a telegraph pole, striking his right knee against the curbstone, which produced a compound comminuted fracture of the patella. The trousers were not torn through, but the underclothing was ground into the wound. He was seen an hour and a half after the injury by Dr. Tiffany, who opened the joint freely, removed the small fragments of patella, and stitched the fibrous capsule. The patient that night became incoherent. The next morning the temperature was 101.5° F., the pulse 118. Delirium occurred in the afternoon. The temperature continued to rise, the pulse became more and more rapid and thready, and the next morning the patient died, less than forty-eight hours after the injury. Gas was detected by the resident physician in the tissues a short distance from the wound a few hours before death, and Dr. Tiffany, within two minutes after death, found by palpation emphysematous crackling in the tissues three inches above the site of the patella, and also in a dusky area over the tibia five inches below the patella. The tissues in these situations were somewhat swollen and oedematous. Upon incision a reddish serous exudate containing gas bubbles escaped. There was no pus. An autopsy was not permitted.

Cultures made by Dr. Mann gave *Bacillus aerogenes capsulatus* with its characteristic reactions. No other organism developed in the anaerobic cultures, and aerobic agar plate cultures were sterile.

This case of emphysematous cellulitis is notable for its extremely rapid and fatal course, death occurring within less than forty-eight hours after the injury. As the outer clothing was not torn, it seems probable that the gas bacillus entered through bits of cloth from the drawers having been ground into the wound. The infection was apparently due exclusively to the gas bacillus.

V. For the following case we are likewise indebted to Dr. Tiffany. At 12.30 a. m. the right forearm of a healthy railway brakeman was crushed between the buffers of two cars and the clothing was ground into the wound. The wound was dressed by two physicians near the scene of accident, and the patient was transferred by train a distance of forty-three miles to Baltimore, where he was seen in the hospital about ten hours after the injury by Dr. Tiffany, who tried to save the limb, which was much mangled. The following morning, thirty-three hours after the injury, emphysematous crackling was noticed about the wrist. The tissues were becoming oedematous. The skin over the doughy crepitating swelling was pale. Two hours later amputation midway between the elbow and shoulder was performed. The ulnar and radial arteries were destroyed. The patient made a good recovery.

Cover-slip preparations and cultures were made both before the operation and immediately after amputation. The cover slips showed bacilli like the gas bacillus and streptococci. Cultures yielded *Bacillus aerogenes capsulatus* and pyogenic cocci. There was also a spore-producing anaerobe which appeared with the gas bacillus in pigeons and guinea pigs inoculated with the cultures of the first generation, but which did not appear in the subsequent cultures and did not grow in sugar agar cultures made from these animals. The gas bacillus gave characteristic reactions in cultures and in inoculated animals.

VI. Dr. Mann has kindly furnished the notes of the following case. Woman, seventy-four years old. When first seen by Dr. Mann on January 18th the hand, forearm, and arm were much swollen, with red lines extending upward from the elbow. The hand was gangrenous, green, with blebs containing bloody offensive fluid. Emphysematous crackling could be felt over the whole extremity as far as to the deltoid muscle. The general condition of the patient was bad, there being high fever, rapid pulse, and delirium. The arm was amputated near the insertion of the deltoid. Examination of the amputated arm showed that the tissues were disorganized and infiltrated with offensive bloody exudate. Gas bubbles were present throughout the tissues and exudate. The patient's general condition was improved the day after the amputation, and the crackling disappeared in the tissues above the line of amputation. Four days after the operation symptoms of tetanus appeared, and death occurred from tetanus on January 24th. No autopsy was obtained.

Cultures made from the amputated arm gave streptococci and *Bacillus aerogenes capsulatus,* which was fully identified. Cultures made from the wound after amputation did not show the gas bacillus.

The preceding six cases belong to the category of gaseous phlegmons, emphysematous cellulitis, or emphysematous gangrene. All were severe infections, three terminating in recovery after amputation and three ending fatally. In only one of the fatal cases was an autopsy performed, and in this gas was not present in the blood and internal organs, although the gas bacillus was cultivated from the lung in Case III.

The following case is inserted in this connection as there was severe phlegmonous inflammation, although gas and the gas bacillus were found only at the autopsy:

VII. Woman, admitted to the service of Dr. Halsted June 24th with phlegmonous infection of the hand and forearm, the joints of the wrist, hand, and fingers being involved. Fever and rapid pulse. June 25th, free incisions into the hand and forearm with sublimate irrigation. The sheaths of the anterior tendons of the fingers contained pus. June 27th, general condition bad; no tendency to wound repair. Offensive odor of the discharges. No emphysema detected. Amputation at the middle of upper arm. Tissues oedematous and vessels thrombosed. Patient died June 30th.

Bacteriological examination of the phlegmon and wound during life showed streptococci and staphylococci. No gas bacilli were detected nor emphysema of the tissues. Cultures from the blood during life were sterile.

At the autopsy, twenty-four hours after death, the spleen was large and soft, the kidneys large, smooth, and pale, being the seat of chronic diffuse nephritis. Sticky purulent discharge covered the exposed surface of the amputation wound. Gas was present in moderate amount in the blood of the heart and vessels; none in the parenchyma of the organs.

Cover slips from the heart's blood showed many bacilli resembling the gas bacillus. Aerobic cultures from the blood, liver, kidney, and lungs were crowded with colonies of *Bacillus coli communis*. Anaerobic cultures from the same situations and from the spleen gave numerous colonies of *Bacillus aerogenes capsulatus* with typical reactions. A pigeon inoculated subcutaneously and into the breast muscle with one cubic centimeter of the heart's blood of a rabbit containing numerous bacilli developed after a few hours an emphysematous swelling, but subsequently recovered.

As the presence of the gas bacillus was not determined during life, its development in the blood with the formation of gas is probably to be regarded as occurring after death. No positive statement can be made as to whether the invasion of the bacillus was through the wound.

We pass now to our observations of a group of cases of perforative peritonitis in which *Bacillus aerogenes capsulatus* was found.

VIII. Woman, admitted to the service of Dr. Osler February 1st with history of pain in the region of the stomach, vomiting, and emaciation extending over three months and increasing in severity during last three weeks. On day of admission she was suddenly seized with a sharp paroxysm of intense pain localized over the stomach. Vomited reddish fluid containing red blood corpuscles. The abdomen became distended, tympanitic, and tender on pressure. The patient passed into a state of collapse and died at 5.20 p. m. on the day following admission to the hospital.

Autopsy fourteen hours after death. The abdomen is greatly distended and tympanitic, and upon incision there is a free escape of gas from the peritoneal cavity. There is a thin fibrinous exudate over the general peritoneal surfaces. The cavity contains about 2,000 cubic centimeters of thin, grayish, turbid fluid holding flakes of fibrin. An infarcted multilocular ovarian cystoma with twisted pedicle nearly fills the pelvic cavity. The intestines are markedly distended with gas. There are multiple necroses in the fat about the pancreas and in the omentum and mesentery. One and a half centimeters below the pylorus there is an ulceration with thickened indurated walls, and 12 millimeters below this a second similar ulceration through all of the coats of the duodenum. The floor of one ulcer is completely closed by adhesion to the pancreas, but that of the other is only partly closed by adhesions, so that there exists here a perforation 5 millimeters in diameter leading into the general peritoneal cavity. Microscopical examination of the thickened edges of the duodenal ulcers showed them to be carcinomatous. The gastrohepatic lymphatic glands are the seat of cancerous infiltration. No metastases in the liver. The rootlets of the portal vein contain small gas bubbles, but no gas bubbles are found in the vessels elsewhere, in the liver or in other parts than those mentioned.

Cover-slip preparations from the fibrinous exudate in the peritoneal cavity showed many bacilli with the morphology of *Bacillus aerogenes capsulatus*. No other bacterial species was found in these preparations. Polynuclear leucocytes were present in only moderate number. Anaerobic cultures from the fibrinous exudate covering the peritoneal surface of the liver gave numerous colonies of the gas bacillus with all of the characteristic reactions. No other species developed. Cultures from the heart's blood sterile. The bacillus isolated from this case was used for numerous experiments on rabbits, pigeons, and guinea pigs. In rabbits subcutaneous inoculation produced gaseous oedema with recovery. For pigeons and guinea pigs the bacillus was highly pathogenic with typical lesions, including the production of peritonitis by intraperitoneal inoculation.

This case of fibrinopurulent pneumoperitonitis following perforation of a cancerous ulcer of the duodenum is of especial interest from the fact that at the autopsy fourteen hours after death, the body having been kept in an ice box, *Bacillus aerogenes capsulatus* was present in large number, and apparently in pure culture in the exudate. Other bacteria, if present at all, were in such small number that they were not detected either in cover-slip preparations or in aerobic or anaerobic cultures. We have occasionally observed in other cases of perforative peritonitis the predominant or apparently exclusive presence of a single bacterial species. Gas bubbles were absent from the blood with the exception of the rootlets of the portal vein. The peritoneal exudate was fibrinoserous without many pus cells.

IX. Service of Dr. Osler. Man, forty-four years old, died July 30th from typhoid fever, on the eighteenth day of the disease, with symptoms of perforative peritonitis. Autopsy five hours after death. The abdomen is greatly distended and very tense. Upon incision there is free escape of gas from the peritoneal cavity. Intestines tympanitic. There is diffuse fibrinous exudate over the visceral and parietal peritoneum with a moderate amount of fluid, which in some places is pus, in other places turbid serum. Thirty-nine centimeters above the valve is a circular perforation, 3 millimeters in diameter, of a typhoid ulcer. There are many ulcers of the ileum, for the most part clean, and the other usual lesions of typhoid fever. There is acute haemorrhagic nephritis and many small abscesses are scattered through the kidney containing typhoid bacilli. Gas bubbles are not present in the blood vessels and viscera.

Cover slips of the peritoneal exudate show various bacteria, including streptococci, bacilli with the morphology of the colon bacillus, slender bacilli, and plump, capsulated bacilli resembling *Bacillus aerogenes capsulatus.* The last species was present in large numbers. *Bacillus aerogenes capsulatus* with other species was obtained from the peritoneal exudate, and from the organs *Bacillus typhosus* (in the bile, bone marrow, mesenteric glands, and kidneys) and *Bacillus coli communis* grew in the cultures.

X. Service of Dr. Osler. Man, died September 9th from typhoid fever in the third week of the disease. Twenty-four hours before death there was severe abdominal pain, followed by symptoms of perforative peritonitis.

Autopsy eighteen hours after death. Abdomen much distended and drumlike. Upon incision of the peritoneal cavity there is violent escape of gas. There is diffuse fibrinous peritoneal exudate. The cavity contains about 1,000 cubic centimeters of turbid fluid mixed with faecal material. There are two small perforations of typhoid ulcers of the ileum. There are many typhoid ulcers, some with necrotic base. The spleen is large and soft. Focal necroses are in the liver. No gas in the heart or viscera.

Microscopical examination of the peritoneal exudate shows the presence of comparatively few cells, but an enormous number of bacteria of various kinds. *Bacillus aerogenes capsulatus* was obtained from the exudate, being present in fair numbers, but the predominating organism was the colon bacillus. There were a few streptococci.

XI. Services of Dr. Osler. Man, died November 26th from typhoid fever, in the fourth week, with symptoms of perforative peritonitis, which developed two days before death.

Autopsy twenty-two hours after death. Abdomen distended, tympanitic, and with beginning greenish discoloration. Gas escaped abundantly on opening the peritoneal cavity. There is extensive fibrinopurulent exudation, most abundant in the lower half of the peritoneal cavity. There is considerable brownish yellow fluid with faecal odor. The serosa is intensely hyperaemic. There are two pin hole perforations of typhoid ulcers in the vermiform appendix, which lies buried in a mass of fibrinous exudate. There are several typhoid ulcers in the small and large intestine. Gas bubbles are present in the inferior vena cava, the systemic and portal veins, and in the right heart.

Cover slips and cultures from the peritoneal exudate gave *Bacillus aerogenes capsulatus, Bacillus coli communis,* and streptococci. The gas bacillus predominated. This same bacillus was found in large number and pure culture in the blood, with the exception of one colony of the colon bacillus in a gelatin roll from the heart's blood. Aerobic cultures from the spleen and liver were sterile.

The three last cases are all examples of pneumoperitonitis due to perforation of typhoid ulcers. *Bacillus aerogenes capsulatus* was mixed with various intestinal bacteria in the exudate, and in such numbers as to indicate its multiplication in the peritoneal cavity, although to what extent this occurred during life cannot be determined. Inasmuch as we have evidence that this bacillus is capable of causing peritonitis, with formation of gas, its presence under these circumstances is not a matter of indifference, even when associated with such recognized pathogenic species as the pyogenic cocci and the colon bacillus. The amount of gas in cases of peritonitis due to intestinal perforation is extremely variable, and it is noticeable that in the preceding cases it was in large quantity in the peritoneal cavity, and that the intestines were markedly tympanitic. The gas found in the heart and vessels in the last case was doubtless referable to post mortem development of the gas bacillus, the autopsy being twenty-two hours after death with evidences of beginning cadaveric decomposition.

XII. Service of Dr. Kelly. Negress, aged thirty-nine, admitted June 26th with strangulated umbilical hernia and faecal vomiting. The strangulation had existed for two days before admission. Temperature 102° F. General condition bad. Herniotomy was performed. Strangulated omentum was excised and the gangrenous gut stitched to the abdominal wall. Death after forty-eight hours.

Autopsy twelve hours after death. Gas in the peritoneal cavity, which is shut off from the artificial anus formed at the umbilicus, this anus being in a loop of intestine 185 centimeters above the valve. Visceral and parietal peritoneum hyperaemic and coated with a thin layer of fibrin. The intestinal loops are adherent to each other and to the omentum by fresh fibrin. Old fibrous adhesions in pelvic peritoneum and over liver and spleen. General arteriosclerosis. Several small old ulcers in the caecum.

Cover slips from the fibrinous exudate on the peritoneum show as the predominating organism *Bacillus aerogenes capsulatus*. In addition a few smaller bacilli and cocci are seen. Cultures furnished *Bacillus aerogenes capsulatus* and also *Bacillus coli communis* and a few streptococcus colonies. Cultures from the blood negative; from the kidney, liver, and spleen, the colon bacillus.

In this case the gas bacillus doubtless entered the peritoneal cavity from the strangulated gut, but whether or not before the operation was determined. The exudate was fibrinous, not purulent. The predominance of the gas bacillus in the exudate at the fresh autopsy indicates that it was a factor in the production of the peritonitis.

The last five cases, together with Case III, afford conclusive proof that *Bacillus aerogenes capsulatus* is sometimes an inhabitant of the intestinal canal, from which it may invade other parts of the body. That it may occur also in the alimentary canal of animals is proved by the following observation:

On the morning of December 10th one of the laboratory stock of rabbits which had not been used for experiment refused to eat and remained quiet in its cage. It was found dead in the afternoon at three o'clock. It was alive an hour before. The body was still warm and not stiff. The rabbit was placed on ice and the autopsy made two hours later. The peritoneal cavity was greatly distended with gas. It contained some blood-stained fluid which had accumulated, especially about the omentum, which was of a deep red color. The stomach was moderately distended with gas, and in its anterior wall was a perforation 2 millimeters in size, through which food extruded. On opening the stomach there were found, in addition to the perforated ulcer, two other circular ulcers, with red edges, one 3 millimeters and the other 4 millimeters in diameter, extending into the submucosa. The intestines were not especially tympanitic. There was no pus in the peritoneal sac. No other lesions were found. There was no gas in the vessels or viscera.

Cover slips from the bloody fluid in the peritoneum showed in large numbers, bacilli with the morphology of the gas bacillus and here and there a coccus. Anaerobic cultures from the fluid yielded numerous colonies of *Bacillus aerogenes capsulatus* with typical reactions and a few colonies of a white staphylococcus.

The occurrence of perforating gastric ulcer as a spontaneous disease in the rabbit is of much interest. The ulcers seemed to be of haemorrhagic origin. The presence at the autopsy, made shortly after death, of a haemorrhagic condition of the omentum and of bloody fluid and of gas in large amount in the peritoneal cavity indicates that the perforation occurred during life. No other cause of death was found. Under these circumstances the large number of gas bacilli, predominating greatly over the bacteria, in the peritoneum is significant. There was no trace of the post mortem digestion of the stomach sometimes found in rabbits.

The following case presents many points of interest, especially the evidence of the production by the gas bacillus of pneumoperitonitis without perforation, of gas in diabetic urine, and of gas cysts in the intestine:

XIII. Service of Dr. Osler. Man with diabetes mellitus and cirrhosis of the liver. Had been ill for nearly two years. Had several profuse haemorrhages from the stomach during the six months preceding death. Developed thrombosis of right femoral vein three months before death. Abdomen became distended and tender. A week before death severe abdominal pain, most marked in right hypochondrium, set in and the abdomen became very much distended. There was a uniform tympanitic sound over the anterior surface of the abdomen. The region of liver dulness was tympanitic. The patient died comatose April 6th. Peritonitis from perforation of a gastric or duodenal ulcer was the clinical diagnosis. Autopsy was made six hours after death. No trace of cadaveric decomposition. Considerable subcutaneous emphysema, especially in the neck and thighs. Heart and blood vessels contain gas bubbles. On opening the distended and tympanitic peritoneal cavity a large amount of gas, free from foul odor, escapes. Intestines moderately distended with gas. Considerable amount of blood-stained turbid serous fluid in the peritoneal sac. No actual pus. Visceral peritoneum hyperaemic. There is a general fibrinous exudate, in most places thin, but thicker over haemorrhagic areas in the intestine. There is haemorrhagic infarction of the jejunum in three places, the largest being twenty-five centimeters in length. Here the coats of the intestine are firm, thickened, and of uniform reddish black color, from infiltration with extravasated blood. There is no perforation of the intestine or stomach. The mesenteric veins are extensively thrombosed, likewise the splenic and portal. There is a collection of cystic cavities filled with gas extending over an area ten centimeters long in the jejunum, which is here not infarcted. These cysts form projections into the lumen of the intestine, some being as large as a bean. They lie in the submucous and mucous membranes. Similar gas cysts are present in the mesentery near its attachment to the intestine. Gas bubbles are present in the omentum. The capsule of the liver is much thickened by diffuse fibrous growth and there is cirrhosis. The urinary bladder is distended with pale, frothy urine due to mixture with gas bubbles. Gas holes are not present in the liver and solid organs, with the exception of the mesenteric glands, which contain small gas vesicles. The endocardium, particularly of the right side, shows diffuse imbibition of blood coloring matter.

Cover slips of the blood show bacilli with the morphology of the gas bacillus. Those from the peritoneum show the gas bacillus in large numbers; in much smaller numbers also bacilli resembling the colon bacillus. Gas bacilli are in apparently pure culture in the various thrombi in the abdominal veins. They are found mixed with other bacteria in the intestine.

Aerobic plate cultures from the heart's blood, lungs, spleen, kidneys, peritoneum, and liver gave colonies of the colon bacillus. Anaerobic plates from the blood, peritoneum, and urine gave numerous colonies of *Bacillus aerogenes capsulatus* with typical reactions. From the intestine the gas bacillus and the colon bacillus were cultivated.

Microscopical examination of sections of the intestine showed extensive invasion by the gas bacillus of the coats in the infarcted areas, where the mucous and submucous coats were necrotic and filled with fibrin, red and white blood corpuscles. Sections through intestine which is not the seat of infarction showed dense masses of gas bacilli upon the surface of the mucosa.

The gas cysts of the intestine occupy both the mucosa and the submucosa. Those which begin in the mucosa and remain there lie next to the muscularis mucosae. Others beginning in the submucosa may involve the mucosa, which is sometimes spread out so as to form a thin film over the surface. The part of the intestine containing these cysts stains poorly, and contains numerous gas bacilli.

In the preceding case a man with diabetes and cirrhosis, who had repeatedly vomited blood, developed extensive thrombosis of the mesenteric, splenic, and portal veins, leading to the haemorrhagic infarction of the intestine. During the last few days of life the symptoms of perforative peritonitis manifested themselves, but the autopsy, made only six hours after death, showed that there was no perforation of the stomach or intestine, although the inflamed peritoneal cavity contained much gas. Gas had begun to make its appearance in the blood vessels, and there was subcutaneous emphysema. Gas cysts were in the intestinal wall, and there was pneumaturia. *Bacillus* *aerogenes capsulatus* had invaded the peritoneal cavity evidently through the infarcted wall of the intestine. In consideration of the symptoms of pneumoperitonitis during life, of the short time which had elapsed between the time of death and the autopsy, of the conditions found at autopsy, and of the experimental results from inoculation of the bacillus, it seems to us altogether probable, if not absolutely demonstrated, that in this instance the gas bacillus was the cause of pneumoperitonitis without the existence of any communication between the peritoneal cavity and an air-containing viscus or the exterior. It is true that *Bacillus coli communis* was also present, although in smaller number than *Bacillus aerogenes capsulatus*, and that the colon bacillus has been claimed, especially in cases of diabetes, as a cause of pneumaturia and gaseous phlegmon, but whatever its status as regards these affections may be, there are no observations which show that the colon bacillus can produce general subcutaneous emphysema, gas bubbles in the blood vessels, or gas in the serous cavities, whereas we have positive evidence of the capacity of *Bacillus aerogenes capsulatus* to cause such conditions as were present in this case. Diabetes mellitus is doubtless a factor favorable to the fermentative production of gas, especially in the urine. Pneumaturia was not observed during life, but it is a symptom which can be readily overlooked when the gas is present in the urine in small amount, and it was not especially looked for.

In the following case the gas bacillus was also associated with nonperforative acute peritonitis, but the evidence as to its causative relation to the peritonitis is not conclusive:

XIV. Service of Dr. Kelly. Negress, sixty years old, with arteriosclerosis, chronic diffuse nephritis, anasarca, and ascites. During last two weeks of life she vomited almost incessantly and developed symptoms of acute peritonitis. Died July 5th.

Autopsy twenty-one hours after death; no greenish discoloration; about 4,000 cubic centimeters of cloudy yellowish red fluid in peritoneal cavity. Visceral peritoneum congested and covered by a thin layer of fibrin. Gas was not noticed on opening the abdominal cavity, but it might have escaped observation. All of the cavities of the heart contain gas bubbles, which are present also in the blood vessels. No subcutaneous emphysema or gas holes in the organs. Diffuse red staining of endocardium and intima. Small contracted kidneys. Sclerosis of the arteries. Old fibrous adhesions about the lungs, liver, spleen, and pelvic viscera. Intestines normal save swelling of the lymphatic follicles.

Cover slips from the heart's blood and peritoneal cavity showed numerous bacilli with the morphology of the gas bacillus which was obtained in culture from these situations and when inoculated into pigeons produced characteristic gaseous phlegmon. Aerobic cultures yielded many colonies of the colon bacillus from the peritoneum and lungs and a few from the spleen, liver, and kidneys.

In the preceding case the possibility that the peritoneal cavity was invaded by the gas bacillus after death cannot be denied. Doubtless the formation of gas bubbles in the heart and vessels was post mortem. As the presence of gas in the peritoneal cavity was not suspected, no especial attention was directed toward its detection. A moderate amount of gas in the peritoneal sac may be readily overlooked, as it escapes on free incision. The peritonitis was characterized by thin fibrinous exudate, turbid blood-stained fluid, and redness of the visceral peritoneum, these being the characters which we have found associated with the presence of *Bacillus aerogenes capsulatus* under conditions indicating the antemortem invasion of this organism. The peritonitis was associated with chronic Bright's disease.

In the preceding fifteen observations (including that of the rabbit) *Bacillus aerogenes capsulatus* invaded the body apparently either directly from without or from the stomach or intestine. In the two following cases it is probable that the bacillus entered by way of the lungs.

XV. Service of Dr. Osler. Woman with acute and chronic valvular endocarditis, chronic Bright's disease, ascites, and pleurisy. Died September 4th. Autopsy twelve hours after death. Peritoneal cavity contains several thousand cubic centimeters of clear straw-colored serum. Peritoneum smooth. Left pleural cavity contains several hundred cubic centimeters of blood-stained serum. Visceral pleura coated with shaggy fibrin. Many subpleural ecchymoses. Left upper lobe and upper part of lower lobe compressed. Remainder of lower lobe solidified by extensive haemorrhagic infarction. The branch of the pulmonary artery supplying left lower lobe occluded by firm reddish gray thrombus, with central softening. Chronic and acute vegetative endocarditis of mitral valve. Hypertrophy and dilatation of heart with antemortem thrombi in right and left auricular appendages. Red, granular kidneys. No subcutaneous emphysema or gas bubbles in the heart, blood vessels, or organs.

Cover slips from the exudate in pleural cavity shows capsulated diplococci and also many encapsulated bacilli with the morphology of *Bacillus aerogenes capsulatus,* which was isolated in culture. The same bacillus was found in the infarcted area in the lung and in small numbers in the bronchial mucus. Aerobic and anaerobic cultures from the blood and other organs were sterile.

The presence of gas bacilli in this case at fresh autopsy in the infarcted lung and pleural cavity, and their absence from the blood vessels and other organs, indicate that this bacillus invaded the pleural cavity from the lung. The consolidation of the lung by haemorrhagic infarction undoubtedly favored the lodgment and survival of the bacillus in this situation. As the presence of gas in the pleural cavity was not suspected, the chest was freely opened at autopsy without taking any precaution to determine its presence. The lanceolate coccus was probably the primary cause of the pleurisy, but in view of Levy's observation,[11] already cited, of the gas bacillus in a case of pneumothorax without perforation, and of our Case XIII of pneumoperitoneum due to the gas bacillus without perforation, it is permissible to suppose that in the present case the gas bacillus may have multiplied in the pleural cavity and produced gas during life, although we can furnish no positive proof of this from the autopsy, and confirmatory evidence from examination of the chest during life is lacking.

XVI. Service of Dr. Osler. Man with thoracic aneurism, aortic insufficiency, and chronic passive congestion with pulmonary infarctions. Died February 14th. Autopsy twenty-four hours after death. Oedema of extremities. Bloody, frothy fluid oozes from nose and mouth. Intestines distended with gas. Peritoneum smooth. In right pleural cavity considerable quantity of blood-stained turbid fluid. Fresh fibrinous exudate over visceral pleura, which is ecchymosed. There are numerous haemorrhagic infarctions in right middle and lower lobes, which contain very little air and a few scattered wedge-shaped infarctions in the upper lobe. The infarctions have a peculiar opaque color and contain gas. The whole lung is congested, and contains abundant frothy, bloody fluid. Branches of the pulmonary artery contain many antemortem thrombi. The left lung likewise contains scattered haemorrhagic infarctions, and presents a condition similar to that of the right. The corresponding pleura is coated with fibrin, but there is little fluid in the cavity. Heart is hypertrophied and dilated; aortic valve insufficient from chronic thickening; marantic thrombi in right auricular appendix and in apex of right ventricle. Aneurism about the size of a small orange involving ascending and part of transverse aortic arch. Atheroma of aorta. Moderate atrophic cirrhosis of liver. No abnormality of stomach or intestine.

The endocardium of the right heart and the intima of the pulmonary vessels and of the veins adjacent to heart are diffusely reddened. A similar reddening does not exist in the left endocardium or the systemic arteries. Gas bubbles are present in the pulmonary vessels, the right heart, the venae cavae. the jugular, femoral, and portal veins. They are absent from the left heart, the systemic arteries, and the abdominal viscera. No subcutaneous emphysema.

Cover slips from the blood in the right heart and the adjacent veins show many bacilli indistinguishable from the gas bacillus, and apparently unmixed with other bacteria. A surprisingly large number of the same bacilli, likewise without other bacteria, are seen in cover slips from infarcted areas in the lung. Cultures from blood of the right heart, thrombi, infarcted lung, and veins containing gas furnished abundant colonies of *Bacillus aerogenes capsulatus.* No other organism was found except streptococci in an anaerobic culture from the heart's blood. Aerobic cultures from blood and organs were sterile. As in other cases, animals were inoculated with the cultures of the gas bacillus which caused the characteristic lesions in guinea pigs and pigeons, and gave the typical reaction when inoculated into the circulation of rabbits which were killed shortly afterward.

Microscopical examination of sections of the lung showed extensive desquamative pneumonia and older and fresh infarctions. The gas bacilli were demonstrated in large numbers in the larger pulmonary vessels and capillaries, in the lymphatics and in the alveoli of the lung. There was considerable infiltration of the margins of the infarctions with polynuclear leucocytes, which often filled up the lymphatics.

It may be mentioned that an autopsy was performed immediately after this one upon a man dying of phthisis and croupous pneumonia within an hour of the death of this patient, and that no gas or gas bacilli were found, so that the latter autopsy may serve as a control. There were no putrefactive changes in either case, the bodies having been kept on ice.

Inasmuch as in the preceding case gas bacilli were found in very large numbers in the pulmonary infarctions, more abundantly than elsewhere, and as gas bubbles were present only in the right heart and the vessels leading to and from this side of the heart, we consider it probable that the invasion of the bacillus in this case took place from the lungs. Invasion from the intestine cannot be positively excluded, but if this had been the direction we should have expected to find development of gas in the liver. Welch and Nuttall demonstrated in their experiments that gas bacilli introduced after death into the right ventricle develop with formation of gas in the course of the vessels leading to and from this side of the heart, and that only after a certain time do they appear in the systemic arteries and the organs. We think it likely, from the conditions found at autopsy, that the bacilli entered the infarcted lung during life, but to what extent they may have multiplied during life we cannot say. Nor can any positive statement be made as to whether the bacilli were conveyed to the lung from without through the air passages or were carried there by the circulation. Inasmuch as the gas bacillus is a not uncommon inhabitant of the intestinal canal, it seems to us probable that, like other intestinal bacteria, this organism may, under certain conditions, enter the circulation without doing any harm. Assuming such an invasion in the present case, it would not be surprising if the bacillus should find in infarcted areas of the lung conditions permitting its survival which do not exist elsewhere. Infarcted areas of the lung are the seat not only of a dense haemorrhagic infiltration, but of actual necrosis of the lung tissue. That the conditions in infarcted lung tissue are sufficiently anaerobic to permit the growth of the gas bacillus can scarcely be doubted when one considers the dark reddish black color of the extravasated blood in pulmonary infarctions. In Case III the gas bacillus seems also to have been localized in the lung, as it was found only there and in the gaseous phlegmon.

In the three following cases *Bacillus aerogenes capsulatus* was found in inflamed parts of the urinary tract:

XVII. Service of Dr. Halsted. Man, admitted December 25th with retention of urine due to urethral stricture following gonorrhoea. During six weeks preceding admission bladder had been catheterized three times daily. General condition septic. Fever, rapid pulse, tongue dry and coated.

Urine contained albumin, casts and pus. Perineal section performed two days after admission. Vesical symptoms relieved, but general condition remained critical, and patient died January 1st.

Autopsy by Dr. Blumer three-quarters of an hour after death in cold weather. Gaping wound in perineum, communicating with bladder, without sign of repair. Chronic purulent cystitis. Double pyoureter and pyonephrosis. Multiple miliary abscesses in the kidneys and the prostate. Stricture of the urethra. Chronic interstitial orchitis. Chronic diffuse nephritis. Arteriosclerosis. Multiple fat necroses in peripancreatic fat, omentum, and panniculus adiposus. Parenchymatous degeneration of heart and liver. A few gas bubbles in the left auricle detected upon first opening the chest.

Cover slips from renal abscesses, renal pelvis, ureters, bladder, and perineal wound showed a large number of bacilli with the morphology of the gas bacillus; also bacilli resembling the colon bacillus, sterptococci, and a few rather long, thin bacilli. Contrary to our custom, cultures were not made at the time of the autopsy, but the organs, wrapped in wet cloths, were put on ice. Culture tubes inoculated the following day did not show the gas bacillus. The demonstration of the gas bacillus in this case, therefore, was not complete, and therefore not satisfactory.

In the following case Dr. Blumer cultivated the gas bacillus from the kidney at the autopsy, and the following day failed to obtain it in culture from the same organ, which had remained during twenty-four hours wrapped in wet cloths on ice.

XVIII. Service of Dr. Halsted. Man, with hypertrophied prostate, admitted December 6th with retention of urine and constant dribbling. Patient was in a stupid condition and no history could be obtained. Died comatose January 1st. Only partial autopsy was permitted. Hypertrophy of prostate. Double pyoureter and pyonephrosis. Multiple miliary abscesses in the kidneys. The renal pelves and ureters were distended with gas and contained pus mixed with bubbles of gas, and small gas cysts were present in the mucous membrane of the pelves, which in places is haemorrhagic and coated with fibrinous exudate. Chronic diffuse nephritis. Spleen large and soft. Gas not present in the blood and organs other than the urinary.

Cover slips from the kidney, renal pelvis, ureters, and bladder showed as the predominant organism bacilli, sometimes encapsulated, with the morphological characters of the gas bacillus. In addition, bacilli resembling the colon bacillus, thin bacilli, and cocci, in pairs and groups, were present in the same situations. *Bacillus aerogenes capsulatus*, with its typical reactions, was isolated in culture from the kidneys and urinary passages.

XIX. For the following case we are indebted to Dr. Williams, of Buffalo, who will publish the report in full in the "Bulletin of The Johns Hopkins Hospital." Through the kindness of Dr. Williams we were enabled to examine cultures from this case, and to confirm his diagnosis of the presence of *Bacillus aerogenes capsulatus:*

The autopsy was made in cold weather, thirty-one hours after death. The pelves and calyces of both kidneys were much dilated and contained pus. There was also double pyoureter. Many small gas cavities were present in the liver (*Schaumleber*) and myocardium. Cover slips from the pus in the renal pelves showed many bacilli like the gas bacillus, and also slender bacilli and streptococci. Gas bacilli and streptococci were found also in cover slips from the liver. *Bacillus aerogenes capsulatus* was isolated in pure culture from the liver and the pus, and the usual inoculations of rabbits, guinea pigs, and pigeons were made with the characteristic results which will be described subsequently.

Although the records of the three preceding cases are not so complete as they might be, they furnish proof of the presence in suppurative inflammations of the upper urinary passages and the bladder of *Bacillus aerogenes capsulatus* in combination with other bacteria. In one of the cases there was gas in the renal pelves and ureters, and it may have been overlooked in the other cases. In one case there was no invasion of the gas bacillus beyond the urinary tract at the autopsy. It is interesting to note the presence of gas cysts in the pelvis of the kidney. The detection of gas bubbles in the left auricle in Case XVII three-quarters of an hour after death is highly significant.

The following case is one of gas cysts of the intestine without invasion of the gas bacillus beyond the intestine:

XX. Service of Dr. Osler. Man in hospital for several weeks with choked disk, headache, and other symptoms of cerebral tumor. There was extremely obstinate constipation. Autopsy January 5th, fifteen hours after death, in very cold weather, the body having been kept in an ice chamber. There was no evidence of post mortem decomposition. There was a vascular sarcoma the size of a hen's egg in the right frontal lobe of the brain. Congestion of lower lobes of the lungs and bronchopneumonia. Chronic diffuse nephritis. The stomach contained dark colored fluid. The large intestine throughout contained hard scybalous masses. The intestines were not distended. The peritoneum was smooth, without excess of fluid. There were two areas in the jejunum, ten centimeters apart, each about five centimeters in length, occupied by gas blebs around the whole circumference of the gut. These cysts are visible through the serous coat as a mass of bubbles, and they form projections on the mucous surface. They occupy all of the coats of the intestine. The individual cysts are the size of a pea and smaller. Upon incision they collapse. There is no evidence of inflammation in the intestine. There is no gas in the other organs or in the blood vessels.

Cover-slip specimens show bacilli apparently identical with gas bacilli among the bacteria in the intestinal contents. Within the cysts the gas bacilli are so numerous that other bacteria are not recognized on the cover slips. Cultures from the intestine and from the cysts gave abundant growth of *Bacillus aerogenes capsulatus*. Cultures from other organs and the blood are sterile.

In the preceding case the invasion of the gas bacillus was limited to the intestinal wall, in which a mass of gas cysts was developed over two circumscribed areas. The bacillus was present in large numbers in the intestinal contents.

The following three cases are examples of the rapid development of gas after death. The first one is of very remarkable character:

XXI. For the report of this case we are indebted to Dr. Berkley, who brought the specimens to the laboratory for our examination. Negro, aged fifty-one, had been an inmate of the epileptic ward of the Bay View Asylum of Baltimore for eleven years. The attacks were of the nature of *grand mal,* and occurred often once or twice daily. On the evening of June 3d he appeared well. At five o'clock the following morning he was found by the attendant unconscious in bed with bubbles of reddish froth issuing from the mouth and nose. He died within twenty minutes of the time that he was found in this condition.

Within six hours after death the body was noticeably swollen and emphysematous. Six hours later the body was still more swollen and emphysematous. and purple blebs of various sizes had appeared over the upper part of the cadaver. The autopsy was held twelve hours after death. No rigor mortis. The body, especially the upper part, is enormously swollen. The eyelids are puffed out and emphysematous. The skin of the scalp is emphysematous. The tissues of the scrotum and penis are greatly distended with gas. The abdomen is enormously distended and tympanitic. The extremities, shoulders, neck, and chest crackle on pressure, and there are large numbers of blebs. A large amount of gas free from putrefactive odor escapes upon opening the peritoneal cavity. The gas burns in jets of bluish flame with detonation on applying a lighted match. The large veins contain blood mixed with gas. The heart is distended with blood and gas bubbles. The blood vessels generally contain gas, but in relatively smaller amount than the subcutaneous tissues. Gas bubbles are present in the liver and spleen in large numbers. On section of the kidneys, blood mixed with gas bubbles oozes from the cut vessels. The lungs are congested, oedematous, of dark red color, and the gas which can be squeezed from the lungs burns with bluish flame. The stomach and intestine are only moderately distended with gas. Cover slips and cultures from all parts showed in very large numbers and in pure culture the gas bacillus which inoculated in pigeons and guinea-pigs caused typical gas phlegmons, not always fatal.

The rapid and diffuse development of gas in the preceding case within a short time after death cannot well be explained, in the light of Welch and Nuttall's experiments, otherwise than as referable to a general distribution of the gas bacilli throughout the body during life by the circulation. No lesions were found to indicate the point of entrance of the bacilli into the circulation. We shall refer to this case again in considering the various conditions with which the gas bacillus is associated.

For the notes of the two following cases we are indebted to Dr. W. T. Howard, Jr., Professor of Pathology in the Western Reserve Medical College of Cleveland.

XXII. Man died in the third week of typhoid fever. Autopsy eighteen hours after death. No cadaveric decomposition. There was no emphysema or marked tympanites at the time of death. Emphysema of the subcutaneous tissues, especially of the neck, chest, axillae, and scrotum, was found at autopsy. The heart and blood vessels generally contained gas. The endocardium and the intima of the vessels were diffusely reddened by imbibed coloring matter. The serous cavities contained gas and blood-stained serum. The mediastinal tissues were emphysematous. The liver and spleen contained gas holes, but were less distended with gas than the kidneys. In the small intestine were typical typhoid ulcers rarely reaching to the muscle. There was no perforation or peritonitis.

The bacteriological examination by cover slips and cultures gave abundant gas bacilli with the usual reactions in cultures and when inoculated into animals.

XXIII. Man died in the second week of typhoid fever. The day before death there was marked tympanites. Autopsy eight hours after death. No odor or discoloration indicating decomposition. There was no swelling of the body, but on careful examination, slight emphysematous crackling was detected on the outer side of the forearm just below the elbow, at the wrists, and along the outer side of the thighs. No gas in the superficial veins. No gas in the heart or blood vessels, or in any organ except the liver, which was large, pale in color, and everywhere perforated with small gas holes. The gall-bladder and the bile-ducts contained a large amount of gas. The walls of the gall-bladder and the surrounding connective tissue were emphysematous. There was extensive necrosis of the liver cells found upon microscopical examination. Peritoneum smooth, without excess of fluid. Many typhoid ulcers in the intestine without perforation.

Cover slips showed an enormous number of gas bacilli in the liver. A few were found in the heart. *Bacillus aerogenes capsulatus* was isolated in culture from the liver, and gave the typical reactions when inoculated into animals.

It is Dr. Howard's opinion—with which we agree—that in the last case the gas bacillus invaded the liver by way of the bile ducts. The large amount of gas in the gall bladder and biliary passages speaks for this view, as we have found that gas is late in appearing in these situations when the gas bacilli are conveyed to the liver by the blood vessels. Although in Case XXII there was far more extensive general development of gas throughout the body, the liver in the present case contained much more gas than in the former. The liver was the only organ which contained gas bubbles, and although gas bacilli had begun to make their appearance in the blood vessels, they had not at the time of the autopsy produced gas bubbles in the blood, although gas had appeared in a few situations in the subcutaneous tissue.

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THE SHATTUCK LECTURE[1]

MORBID CONDITIONS CAUSED BY *BACILLUS AEROGENES CAPSULATUS*

Mr. President and Fellows of The Massachusetts Medical Society.—Although the subject which I have chosen for this lecture relates for the most part to infrequent affections, the scientific and practical interest attaching to them is considerable and varied. Many instances of the presence of free gas in parts of the body where it does not normally occur and in association with various diseases were recorded by writers of past centuries and were even then the subject of much speculation. The discussion turned generally around the question whether the gas was atmospheric air or the result of putrefaction—a question which in most cases could be solved only by bacteriological examinations. The most numerous and important of such examinations have been made during the last decade, and, although these have left problems still unsolved, they have corrected many current errors and have shed a flood of light upon conditions which were formerly among the most mysterious in pathology.

While it has been demonstrated that various bacteria may be concerned in producing gaseous affections, it is now evident that the bacillus which I discovered in 1891 and to which I gave the name *Bacillus aerogenes capsulatus* is the one whose causative agency is best established and most frequently in action. What I shall say will relate mainly to this microorganism and its pathogenic effects.

Historical.—As a certain amount of confusion concerning the dates of the first publications[2] on this bacillus exists in foreign literature on this subject, it may be well to state that I reported my observations in November, 1891, to The Johns Hopkins Hospital Medical Society and that the full report of these observations and of the characters of the bacillus was published in July-August, 1892, by Dr. Nuttall and myself.[3] E. Fraenkel's first publication[4] was a short preliminary one which appeared in January, 1893, and was followed in the same year by his valuable monograph on gaseous phlegmons.[5] In August, 1893, one year after the publication of the paper by Nuttall and myself, appeared simultaneously the interesting articles of P. Ernst[6] and of Graham, Steward and Baldwin.[7] Early in 1894 Mann published from my laboratory an observation of emphysematous gangrene caused by *Bacillus aerogenes capsulatus*[8] and in January, 1896, Dr. Flexner and I published an extensive paper reporting twenty-three human cases, including not only six personal observations of emphysematous gangrene but also examples of submucous gas cysts, pneumoserositis, and various other pathogenic manifestations of this bacillus.[9] In July, 1895, appeared Goebel's preliminary communication[10] and in the following year his full paper on the bacillus of foamy organs.[11] Of the subsequent records the most numerous and valuable have appeared in this country, although they appear to be little known to most European writers.[12] I shall have occasion to refer later to many of these publications.

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[1] Delivered before the Massachusetts Medical Society, Boston, June 12, 1900. Boston M. & S. J., 1900, CXLIII, 73-87.

[2] Levy's description in 1891 (Deutsche Ztschr. f. Chirurg., XXXII.) of "kleine, feine" bacilli, cultivated from a gaseous abscess and growing in long threads and chains only at body temperatures and cultivable only in the first generation, without animal experiments, cannot be accepted as an identification of *B. aerog. capsulatus*, or indeed be readily reconciled with its characters.

[3] Bulletin of The Johns Hopkins Hospital, 1892, III., p. 81.

[4] Centralbl. f. Bakter., XIII., p. 13.

Nomenclature.—Dr. Fraenkel has kindly favored me with cultures of the bacillus which he cultivated from gaseous phlegmons and to which he gave the name *Bacillus phlegmones emphysematosae.* There can be no question whatever as to the identity of his bacillus with our *Bacillus aerogenes capsulatus,* a point upon which we are both agreed, and which is also made certain by Goebel's studies under Fraenkel's supervision. According to the generally accepted principles of the nomenclature of zoological and botanical species the latter name, as being the first one applied, should be preferred to the former. It is, moreover, as pointed out by Muscatello, not open to the objection of implying exclusive relationship to a single disease, as is the case with Fraenkel's designation of the bacillus. As a matter of fact the capacity to produce gaseous phlegmons is only one of many pathogenic manifestations of *Bacillus aerogenes capsulatus*. Unfortunately I think that we both erred against the canons of botanical nomenclature in using a trinomial rather than a binomial name for a species.[13]

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[5] Ueber Gasphlegmonen. Hamburg u. Leipzig, 1893.

[6] Virchow's Archiv., CXXXIII., p. 308.

[7] Columbus Med. Journ. XII., p. 55.

[8] Annals of Surgery, XIX., p. 187.

[9] Journal of Experimental Medicine, 1896, I., p. 5.

[10] Centralbl. f. allg. Path. u. path. Anat., VI., p. 465.

[11] Jahrb. d. Hamburg. Staatskrankenanstalten, IV.

[12] Thus v. Hibler in 1899 (Centralbl. f. Bakt. XXV., p. 513 et seq.) in an elaborate study of pathogenic anaerobes is entirely ignorant of our work and that of other American investigators on *B. aerog. capsulatus.* The information of Hitzschmann and Lindenthal (Sitzungsb. d. K. Akad. d. Wiss., Math.-Naturw. CL., Wien., 1899,) on the American work is secondhand and both incomplete and inaccurate, in these respects being in unfavorable contrast to that of Muscatello and Gangitano (Riforma Med., 1900, II. p. 508, et seq.) who write also on the subject of emphysematous gangrene. Knowledge of *Bacillus aerogenes capsulatus*, under the name *Bacillus perfringens*, has begun to appear in France in the last two years, but without any evidence of acquaintance with the American publications.

Characters of Bacillus.—Since our first publication only one material addition has been made to the extended description given by Nuttall and myself of the morphological and cultural characters of *Bacillus aerogenes capsulatus* or the gas bacillus, as I shall briefly call it.[14] Fraenkel noted the presence of spores in a few of the bacilli growing in one lot of agar containing sodium formate, and in 1897 Dunham[15] observed spores in blood serum cultures, but not in other media.

Our further studies of the gas bacillus obtained from different sources have shown a moderate range of variations in some of its properties. This is true especially of spore-formation, rapidity of liquefaction of gelatine, presence of capsules, retention of Gram's stain, and virulence. While some specimens of the bacillus seem never to form spores on any culture medium, others, and these appear to be the more common, do so occasionally, especially upon blood-serum, in mannite bouillon, and on plain agar. In animals inoculated with pure cultures we have not observed spore-bearing bacilli.

As a rule the bacillus liquefies gelatine slowly, but some specimens do so scarcely at all, and others with fair rapidity.[16]

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[13] Migula, who, with considerable success, has attempted to reform bacteriological nomenclature, has given the binomial names "*Bacterium welchii*" to *Bacillus aerogenes capsulatus* and "*Bacterium emphysematosum*" to *Bacillus phlegmones emphysematosae* (System der Bakterien, II., pp. 392 and 383). He is, however, in error in describing this organism under two different names, as his *Bacterium welchii* and *Bacterium emphysematosum* are identical.

[14] As regards these characters, it will suffice here to say that the microorganism is a rather coarse, nonmotile, anaerobic bacillus, staining by Gram, growing on all of the ordinary culture media under anaerobic conditions, best at body temperature, but also at room temperature, forming spores inconstantly according to the race and the culture medium, and capable of forming gas, not only by fermentation of sugars, but also from proteids. The full description of the characters may be found in Welch and Nuttall's paper. The gas, according to Dunham's analyses, is composed approximately of 64 per cent hydrogen, 28 per cent carbon dioxide, and 8 per cent of a residual gas believed to be mainly nitrogen. It has no foul odor.

[15] Bulletin of The Johns Hopkins Hospital, 1897, VIII., p. 68.

[16] In our original communication we noted peptonization and softening of the gelatine, but this was so slow and slight that we then preferred to class the bacillus among the nonliquefiers. Further experience has shown that the bacillus is a liquefier, but generally a slow one.

As stated in our original article capsules are not constantly present, but I have generally found no difficulty in demonstrating them in the situations and by the method described by us, and, with the exception of Hitschmann and Lindenthal, most other investigators have been able to demonstrate capsules, when these are searched for by suitable methods.

While the bacillus is to be ranked among those which stain by Gram, it is sometimes rather noticeable in cover-slips from cultures that among well stained bacilli others are partly or wholly decolorized, and this may be observed in members of a single chain. In the tissues the bacillus stains well by Gram. Differences in the viability of cultures were pointed out in our first paper.

It sometimes happens that original cultures from human beings show only a feeble growth, with relatively weak power of gas-production, while subsequent cultures, especially those obtained after passage through the animal body, present the usual vigorous growth and other typical characters. Pratt and Fulton[17] from a typical case of foamy organs, with gas throughout the body, were unable to cultivate the bacillus at all, although twelve anaerobic culture tubes containing various media were inoculated from different parts of the body. This negative result they attribute to the fact that the body had lain in a cold storage vault for sixteen hours after death, an explanation which has some support in an observation previously reported by Welch and Flexner, but still seems hardly satisfactory, as under similar conditions the bacillus has been often cultivated.

Lactose, glucose and saccharose are all fermented by the gas bacillus, the first with the largest production of gas and the last with the smallest. There is apparently no fermentation of mannite, at least the gas is not appreciably more than in sugar-free media. The amount of hydrogen greatly preponderates over that of carbon dioxide.

E. Fraenkel in 1893 was the first to demonstrate the etiological relation of the gas bacillus to gaseous phlegmons, our previous investigations being concerned mainly with the so-called foamy organs (Schaumorgane) and the presence of gas in the blood, our results being confirmed a year later by P. Ernst. Soon after Fraenkel's publication we were able to confirm his discovery of the causation of gaseous phlegmons by *Bacillus aerogenes capsulatus,* and to repeat with similar results his animal experiments.

Material or cultures fresh from the infected body are usually highly virulent for guinea pigs, pigeons and sparrows (E. Fraenkel), which succumb to a rapidly spreading local necrosis of the tissues with abundant development of gas, the bacilli invading the blood during life only in small number or not at all. There is more or less bloody oedema, but otherwise little inflammatory reaction, leucocytes being present usually only in small numbers in the exudate. Rabbits and mice, while not wholly immune, are far less susceptible than guinea pigs and pigeons. Dr. Lanier in my laboratory in 1896 succeeded in producing typical gaseous phlegmons around the fractured bones of rabbits inoculated intravenously with pure cultures and Muscatello has obtained the same results. There are considerable differences in the degree of virulence of the bacillus even in fresh cultures, and old ones may be of very slight virulence.

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[17] Boston Med. and Surg. Journ., June 7, 1900, p. 599.

One of the most interesting and valuable tests of the gas bacillus is its power of producing gas abundantly in the blood, organs and tissues of rabbits killed a few minutes after intravenous injection, a power not possessed by colon bacilli. The differential value of this test is as great as that of cultures in fermentation tubes. The blood and tissues of the dead rabbit make the culture medium, the body of the animal takes the place of the test tube, the inoculation is an aseptic one, the bacteria are spread by the blood current and the conditions are anaerobic. This procedure, which was introduced by Nuttall and myself and has been fully described by us, we have found useful in isolating the bacillus, in separating it from other bacteria which may resemble it, and the demonstration of one of its most fundamental characteristics, namely, the power to produce gas from proteid material.

Among the points distinguishing the bacillus of malignant oedema from *Bacillus aerogenes capsulatus* may be mentioned the following: The malignant oedema bacillus is somewhat thinner, has greater tendency to grow into filaments, is less readily stained by Gram, produces spores regularly in culture media, is motile, liquefies gelatine more rapidly, produces a foul odor, produces less gas in lactose bouillon, peptonizes clotted casein, generates little or no gas in rabbits inoculated intravenously and then killed, and by subcutaneous inoculation in susceptible animals causes spreading bloody oedema with little or no development of gas bubbles, and appears after death in filaments on serous surfaces.

The bacillus to which Lindenthal[18] has quite unnecessarily given the name *Bacillus emphysematis vaginae* is doubtless identical with *Bacillus aerogenes capsulatus*. The same is true of Veillon and Zuber's[19] *Bacillus perfringens*, found by them in appendicitis, by Guillemot[20] in gaseous gangrene and by Soupault and Guillemot[21] in gaseous abscesses, and of Buday's[22]

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[18] Wiener klin. Wochensch, 1897, p. 3, et seq.

[19] Arch. de méd. exp., 1898, X, p. 539.

[20] Compt. rend. Soc. de biol. 1898, 10 s., V, p. 1017.

[21] Bull. et mém. Soc. méd. d. hôp. de Paris, 1900, 3 s, XVII, p. 216.

[22] Centralbl. f. Bakt., 1898, XXIV, p. 369.

Bacillus cadaveris butyricus, found in foamy organs. I am strongly inclined to the opinion that the anaerobic bacillus isolated by Achalme and others from several cases of acute articular rheumatism and found by Savtchenko and Mielkich in the soil is likewise identical with our gas bacillus.[23]

Distribution.—The surmise expressed by Welch and Nuttall that the gas bacillus is widely distributed in nature has since been confirmed. The natural habitat of the organism is the intestinal canal and the soil, the home of so many other anaerobic bacteria. Welch and Flexner in 1896 brought evidence of the presence of the bacillus in both of the situations mentioned. Clopton of The Johns Hopkins Hospital has found the bacillus twice in the appendix vermiformis. Howard[24] has recently reported the presence of morphologically identical bacilli in the intestines of twenty-five consecutive human cases examined post mortem, and in ten of these he demonstrated the bacillus by cultures and inoculation of animals. The same conclusion has been reached by Hitschmann and Lindenthal. The gas bacillus has been repeatedly cultivated from the intestine in my laboratory, but we have made no systematic study of the frequency of its presence. I have found the bacillus also in the intestines of rabbits, dogs and swine, and here it is interesting to note the frequency with which submucous gaseous blebs are found in the pig's intestines at autopsy.

In 1896 Dr. Walker, at The Johns Hopkins Hospital, succeeded in finding the gas bacillus in dust collected by sweeping floors, proving its presence both by cultures and animal experiments. My assistant, Dr. Harris, has cultivated the bacillus from the contents of an old cesspool. I had previously reported in 1896 the isolation of the bacillus from a bullet removed from the head of the tibia in a case of gaseous phlegmon, and E. Fraenkel[25] has cultivated the gas bacillus from a splinter of wood extracted from a wound in a case of tetanus. These observations confirm the natural inference to be drawn from the study of cases of traumatic emphysematous gangrene, in most of which the source of infection is manifestly foreign material, especially dirt, in wounds. In the light of these demonstrations of the wide distribution of the gas bacillus in the outer world and in faeces, the conclusion is warranted that it must occasionally be present upon the human skin.

We are not informed whether there are differences in the regional distribution of the gas bacillus. The fact that during the last decade a larger number of cases of emphysematous gangrene have been reported from Baltimore than from any other single locality is due probably to our interest in the subject and consequent search for cases. The bacillus has been found not only in America and Europe, but Dr. Flexner has brought back reports of three infections with the gas bacillus in Manila, observed during a stay of three months.

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[23] Achalme, Ann. de l'Inst. Pasteur, 1897, XI p. 845; Pic and Lesieur, Journ. de phys. et de path. gén., 1899, I, p. 1007, and Savtchenko and Mielkich, Arch, russes de path., 1899, VIII, p. 145.

[24] Contributions to the Science of Medicine dedicated by his pupils to William Henry Welch, on the 25th Anniversary of his Doctorate, p. 461, Baltimore, 1900.

[25] Münch, med. Woch., 1899, No. 42 and 43.

Gas Bubbles in the Blood and Organs

We turn now to the consideration of the various conditions in which the gas bacillus has been found in human beings. We need not pause to consider the presence of this bacillus in ordinary cadaveric decomposition, a circumstance sufficiently explicable by the occurrence of this organism in the healthy intestinal canal.

Of an entirely different nature are the cases in which gas bubbles are found in the blood and organs within a few hours after death and without any trace of ordinary putrefaction. Such a condition has been recognized at autopsies as soon as one, two, three, five, eight hours after death. It may occur not only after death from gaseous phlegmon, when, however, it is by no means constant, but also after death from the most varied causes. It has been observed repeatedly in autopsies on pregnant and puerperal women, especially after death from abortion and acute sepsis.

There is every graduation from cases with a few bubbles of gas in the blood or tissues to those with extensive emphysema of the organs and tissues. The term "foamy organs" (Schaumorgane of the Germans) may be applied to the latter condition. The liver is the organ most frequently the seat of early and abundant development of gas, but there is no definite rule as to the distribution and amount of gas in different cases. As will be explained later, the invasion in the majority of cases is from the intestine. That the gas bubbles may be dislocated from their original position in liquid and soft material in the body is self-evident, but I have not found them unassociated with gas bacilli.

Formerly this early presence of free gas in the heart and vessels, without evident post mortem decomposition, was very generally explained by the assumption of entrance of air into the circulation, even when no portal of entry could be found.[26] The most extensive application of this explanation was made in the pregnant and puerperal cases. It is remarkable that the first case of this nature to be examined bacteriologically was that reported by me in 1891. In this and in all subsequent similar cases with satisfactory bacteriological examination *Bacillus aerogenes capsulatus* was found.

[26] Cless. Luft im Blute. Stuttgart, 1854, and Couty. Thèse, Paris, 1875.

The main questions which arise concerning the interpretation of these cases are whether the invasion of the bacilli and whether the development of the gas are ante mortem or post mortem phenomena.

Rabbits survive the introduction of large numbers of gas bacilli directly into the circulation, unless there exists somewhere in the body necrotic or damaged tissue offering little or no vital resistance. If the animal be killed within a few minutes after the intravenous injection of the bacilli and kept in a warm place, there are abundant multiplication of the bacilli and large development of gas throughout the body within the space of six or eight hours; whereas if the bacilli be introduced at one point, as for example the right heart, of a rabbit just killed, it takes a much longer time, often twenty-four or forty-eight hours, for gas and bacilli to make their appearance at points far distant from the seat of inoculation. It seems justifiable to draw from these three groups of experiments, which have been fully reported by Welch and Nuttall, the conclusion that when bacilli and gas are found within a few hours after death widely distributed in the body, the gas bacilli have entered the circulation during life, but probably in most cases only shortly before death.

There is one factor, however, to be considered which is absent in the experimental cases and may be present in human beings, to wit, the quick disappearance of the bactericidal power of the blood. This factor is an important determinant of the rapidity of onset of post mortem decomposition. Very soon after death from certain diseases, and particularly from snake venom, bacteria may make their appearance in the blood and organs. An explanation of these cases is furnished by the experiments of Ewing and myself,[27] which demonstrated that the blood of rabbits killed by rattlesnake venom is practically devoid of bactericidal power so that immediately after or even shortly before death bacteria can start growing in the body as they would in a tube of beef-broth. But after all due allowance has been made for the possible reduction or loss of bactericidal power of the blood, I still consider that it is not possible to explain some of the cases in which bacilli and gas have been found in the heart, blood vessels and organs very soon after death, especially when the corpse has been kept in a cold place, otherwise than upon the assumption of the distribution of the bacilli by the circulating blood.

It is another question whether gas as well as bacilli may be present in the circulating blood and internal organs during life in the class of cases now under consideration, and I regret to be unable to furnish a positive answer to this question. I do not see how an affirmative answer can be obtained otherwise than by the actual demonstration of gas in these situations either during life or immediately after death. Gas bubbles and bacilli have been found in the heart and vessels within an hour after death, but that is time enough for bacilli which have already been introduced to multiply and to begin to form gas. I at first thought that absence of nuclear staining around the gas bubbles and masses of bacilli might serve as an indication of their presence during life, and this view is advocated by P. Ernst, but I have since learned from experiments on rabbits that this is not a decisive criterion, although often both in rabbits and in human beings there is no defect in nuclear staining around the bacilli and gas bubbles.

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[27] Lancet, 1894, I, p. 1236.

I know of no other pathogenic microorganism which offers such difficulties in determining whether its effects in the interior of the body have been produced before or after death. The difficulty arises from the circumstance that these effects in most cases and most situations consist almost entirely in local necrosis and formation of gas, whether the invasion and growth of the bacilli be before or after death, and that unlike most pathogenic bacteria the gas bacillus grows better in the dead than the living body. Possibly some importance in the solution of the problem may attach to the demonstration of emboli of liver cells and of bone-marrow cells which were in enormous numbers in the pulmonary vessels in a case of gaseous phlegmon of the submammary tissues following infusion of salt solution. At the autopsy made by Dr. Carroll the liver and other organs were emphysematous. Further observations with reference to these emboli in this class of cases are needed.

I do not consider that there is any inherent improbability in the supposition that gas bubbles may be in the circulating blood during life without causing speedy death from gaseous embolism. It is only when a large volume of air is introduced quickly into the blood current that sudden death results from air embolism. Very exaggerated ideas have prevailed among physicians as to the dangers from the entrance into the circulation of small quantities of air. Laborde and Muron[28] injected into the external jugular vein of a dog 1120 c. c. of air in the space of one hour and a half without causing death, and Jürgensen[29] into the left femoral artery of a narcotized dog weighing 43½ kilo 3,650 c. c. in the space of two hours and twenty-five minutes with only slight disturbance of the respiration and of the action of the heart. Hare[30] on the basis of experiments likewise controverts current beliefs concerning the dangers from entrance of air into the veins.

I have come across in the older literature, from the days when venesection was a common practice, reports of cases in which blood containing bubbles

[28] Comptes rend. Soc. de biol., 1873, V.

[29] Deutsches Arch. f. kl. Med., 1882, XXXI, p. 458.

[30] Therap. Gazette, 1889, 3 s, V, p. 606.

of gas escaped during venesection from veins of the arm.[31] In none of these was there evidence that air had gained entrance to the circulation. Maisonneuve,[32] in incising two gaseous phlegmons of the thigh following compound fracture, observed the escape of blood containing gas bubbles from the cut veins, and was able to trace the gas within the veins for a long distance.

It seems to me very improbable that an anaerobic bacillus, such as the gas bacillus, can multiply in the circulating blood, still this bacillus is less sensitive to the presence of oxygen than many anaerobes, and we do not know whether the loose combination in which oxygen is present in the blood would necessarily prevent its growth under all circumstances.

I see no reason why this bacillus might not multiply in the liver, spleen and most other internal organs, as we know it can do in parts open to inspection during life. We have positive evidence in the cases reported by Graham, Steward and Baldwin, and by Dunham that gas bacilli may be conveyed by the circulation from an infected portal of entry, in the one case the puerperal uterus and in the other a urethral wound, to distant parts of the body, and there produce subcutaneous emphysema and necrosis. There is no part of the body which offers such favorable conditions for the post mortem growth of the bacillus as the liver, probably on account of its content of carbohydrate, and, if the liver like the integuments were open to inspection during life, I believe that we should find evidence that in certain cases the emphysema of this organ, which is such a conspicuous post mortem phenomenon in instances of invasion by the gas bacillus, had begun during the life of the patient. Emphysema of mucous membranes, open to inspection, we know can exist during life. This subject will be considered subsequently.

In the great majority of the instances, however, in which gas bubbles are found in the blood and internal organs at autopsy, the evidence is in support of the view that the development of the gas is a purely post mortem phenomenon. Certainly the greatest caution should be exercised in the interpretation of any such cases as vital processes, even in early autopsies without ordinary putrefaction. One thing which our investigations have established is that the finding of gas bubbles in the blood vessels and heart within as short a time as one hour after death, furnishes in itself no proof of the entrance of air into the circulation. I shall refer later to the question of gaseous embolism in cases of emphysematous gangrene and of physometra.

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[31] Marshall's case reported by May, Trans. Path. Soc., London, 1858, IX, p. 157; Durand-Fardel's case also cited by May, and Pirogoff's case in his Grundzüge d. allgem. Kriegschirgie, p. 1063, Leipzig, 1864.

[32] Cited from Hitschmann and Lindenthal, *loc. cit.*

Emphysematous Gangrene

In a few instances we have found in wounds, usually compound fractures or gunshot injuries, in which dirt had gotten in, *Bacillus aerogenes capsulatus* without the presence of gas or other evidence that the bacillus was producing any characteristic effects.[33] Such cases have always been watched by the surgeons with anxiety, and it is probable that at least in some the early recognition of the bacillus, followed by free incisions and thorough cleansing and disinfection, has warded off a subsequent grave infection. In view of the wide distribution of the gas bacillus in the outer world and in the intestinal contents, it is probable that it must not so very infrequently gain access to wounds without securing a foothold. While this innocent behavior, with which we are also familiar in the case of the tetanus bacillus, may sometimes be due to attenuated virulence of the bacillus, it is probably oftener attributable to accessory circumstances, such as resistance of the patient, the condition of the wound and surrounding tissues, and lack of association with other microorganisms and foreign substances.

It is as a cause of that most dreaded of wound complications, emphysematous gangrene, that *Bacillus aerogenes capsulatus* especially claims the interest of surgeons. The classical clinical descriptions of this disease we owe to Maisonneuve[34] and to Pirogoff,[35] the former giving to it the name "gangrene foudroyante," and the latter designating it "primary mephitic gangrene" or "acute gangrenous oedema." Among other more or less common designations are "emphysematous or gaseous gangrene," "gaseous phlegmon," "septic emphysema," "érysipèle bronzé" (Velpeau), "progressive gangrenous oedema," "gangrenous septicaemia," and "emphysematous cellulitis."

This wound complication was more common in preantiseptic times, especially in military surgery, than it is today; but at least seventy cases have been reported during the last quarter of a century. In prebacterial days the affection was attributed by some writers to the penetration of air into the tissues, but by most to the decomposition of the tissues, particularly of adipose tissue and bone-marrow, brought by an injury into contact with the atmosphere.

Bottini,[36] in 1871, was the first to demonstrate the infective nature and transmissibility of emphysematous gangrene. Later, Gussenbauer also recognized the disease as a definite infection, and attributed it to the bacteria of putrefaction. After Pasteur's discovery in 1877 of his "*Vibrion septique,*" more commonly, since Koch and Gaffky's investigations, designated *Bacillus oedematis maligni,* and especially after Chauveau and Arloing's[37] paper in 1884, cases of emphysematous gangrene have been usually reported, especially in France, as instances of Pasteur's gangrenous septicaemia or Koch's malignant oedema. W. Koch's[38] attempt to identify the disease with symptomatic anthrax (Rauschbrand) was based on faulty bacteriological studies and has met with no confirmation. In 1884 F. J. Rosenbach[39] reported finding in cover-slip specimens from two cases of traumatic emphysematous gangrene coarse bacilli, some of which had terminal spores. These he was unable to cultivate, only aerobic methods being employed. It is probable that Rosenbach saw the gas bacillus in these cases, but without distinguishing it from associated spore-bearing bacilli.

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[33] Such cases have been reported by Bloodgood from The Johns Hopkins Hospital in Progressive Medicine, December, 1899, IV, p. 158.

[34] Gaz. méd. de Paris, 1853, p. 592.

[35] Grundzüge d. allgem. Kriegschirurgie, pp. 867 and 1006, Leipzig, 1864.

[36] Gior. d. r. Accad. di med. di Torino, 1871, 3 s. X., pp. 1121 and 1138.

A critical examination of the records of alleged malignant oedema in human beings shows that in very few was the organism concerned satisfactorily identified as the genuine malignant oedema bacillus. Very often it has been simply assumed without more than a microscopical examination that bacilli found in spreading oedematous conditions with or without gas have been those of malignant oedema, and even where cultures and animal experiments have been employed the descriptions are frequently so meagre as to leave the identity of the organism wholly in doubt. In France it is usually assumed without any discussion and even without any bacteriological examinations that gangrène foudroyante is malignant oedema (Pasteur's septicaemia),[40] and the same ignorance of the present status of this subject is still sometimes encountered in England, Germany and elsewhere. Nevertheless the investigations of the last seven years, beginning with those of E. Fraenkel and soon followed by observations of myself and collaborators, have demonstrated that by far the most common and important specific cause of gaseous phlegmons or emphysematous gangrene is *Bacillus aerogenes capsulatus.*

Whether the bacillus of malignant oedema can produce an identical or similar anatomical and clinical affection in human beings I regard as an unsettled question. It is certainly remarkable in view of current doctrines

[37] Bull. Acad. de Méd. 1884, 2 s., XIII, p. 604.

[38] Deutsche Chirurgie, Lief. 9, Stuttgart, 1886.

[39] Die Mikro-organismen bei den Wund-Infections-Krankheiten des Menschen, Wiesbaden, 1884.

[40] An exception is Guillemot (Comptes rend. Soc. de biol., 1898, 10 s., V, p. 1017) who has found *B. aerog. caps.* in a case of gaseous gangrene and who controverts the prevalent view of authors who attribute this disease exclusively to Pasteur's vibrio.

in text-books that neither E. Fraenkel nor I with our relatively large experience, nor indeed, so far as I am aware, anyone who has made himself thoroughly acquainted with *Bacillus aerogenes capsulatus,* has encountered an instance of emphysematous gangrene in man caused by the bacillus of malignant oedema. The whole subject of human malignant oedema is one which needs thorough revision and investigation by more exact bacteriological methods than have yet been applied to it.[41] I have already mentioned the chief points of difference between the bacillus of malignant oedema and *Bacillus aerogenes capsulatus.*

There is a relatively small group of cases of gaseous phlegmon attributed by those reporting them[42] either to the colon bacillus or the proteus bacillus. In most of these cases anaerobic culture methods were not employed. No one has succeeded in producing experimentally gaseous phlegmon with either of these bacilli, and I think there is good reason to be skeptical concerning their capacity to produce this disease, unless perhaps *Bacillus coli* may do so in diabetics.

It is possible that some of those reporting the colon bacillus as the cause of emphysematous gangrene may have confounded with it a facultative anaerobic bacillus which we have isolated from two cases of this disease, and which has been studied in my laboratory by Dr. Lanier. It resembles in anaerobic cultures very closely *Bacillus aerogenes capsulatus,* but it is capable of aerobic growth also, and the rods are thinner and more like colon bacilli. It has the power of producing gas abundantly in the blood and tissues of rabbits killed a few minutes after intravenous injection, a power not possessed by genuine colon bacilli. I have already spoken of the importance of this test, which has been employed by none of the writers who have claimed to find colon bacilli as the cause of gaseous phlegmons. This bacillus, when virulent, is capable of causing the same spreading and fatal emphysematous necrosis in guinea pigs and pigeons as in *Bacillus aerogenes capsulatus.*

I have been accustomed to speak of this bacillus, to which I have called attention in previous publications, as the aerobic variety of our gas bacillus.

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[41] In the case reported recently by Brabec (Wiener klin. Rundschau, 1900, XIV, pp. 145 and 167) the identification of the malignant oedema bacillus seems satisfactory. Here there was extensive bloody oedema without gas, so that the case was not one of emphysematous gangrene. On the other hand Hämig and Silberschmidt (Correspondenzbl. f. Schweizer Aerzte, 1900, XXX, p. 361) bring no proof of any consequence that they were dealing, as they supposed, with the malignant oedema bacillus in two cases of gangrène foudroyante.

[42] Chiari, v. Dungern, Bunge, Klemm, Hlava, Evans, Grassberger, Hauser, Margarucci, Muscatello, Hitschmann and Lindenthal.

I believe, however, that it is identical with Sanfelice's *Bacillus pseudooedematis maligni*[43] with which he is inclined to identify Klein's "new bacillus of malignant oedema."[44] Chavigny[45] has isolated apparently the same bacillus, which he likewise identifies with Sanfelice's *Bacillus pseudooedematis maligni,* (not to be confounded with the pseudooedema bacillus of Liborius), from a case of gaseous gangrene, and he also calls attention to the probability that others may have mistaken it for the colon bacillus. While, therefore, unwilling upon existing evidence to accept the colon bacillus as a demonstrated cause of gaseous gangrene (except perhaps in diabetics), I am of the opinion that an aerobic bacillus probably identical with Sanfelice's *Bacillus pseudooedematis maligni,* is capable of producing this affection, but is much less frequently concerned than *Bacillus aerogenes capsulatus.*

I have collected forty-six cases of emphysematous gangrene, in all of which *Bacillus aerogenes capsulatus* was demonstrated, and, therefore, all reported or observed during the last seven years.[46] This is a far larger number of cases than has ever been brought together before.

Thirty-two are reported by American observers and only fourteen by foreign investigators. Of the former group of cases sixteen were observed in

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[43] Ann. d. Istit. d'Igiene sper. d. Univ. di Roma, 1891, N. S., I, p. 365, and Ztschr. f. Hyg. 1893, XIV, p. 352.

[44] Centralbl. f. Bakter., 1891, X, p. 186.

[45] Ann. de l'Inst. Pasteur, 1897, XI, p. 860.

[46] This list includes 16 cases observed in Baltimore, mostly at The Johns Hopkins Hospital, of which 2 are unpublished, and the remaining 14 have been published by Mann (1), Ann. Surg., 1894, XIX, p. 187; Welch and Flexner (6), Journ. Exp. Med. 1896, I, p. 5; Martin (1), University Bulletin, 1896, I, No. 3, and Bloodgood (6), Progressive Medicine, 1899, IV, December, p. 158. The notes of an additional unpublished case observed in Manila have been given me by Dr. Flexner. There are also 3 unpublished cases for the records of which I am indebted to Dr. Carroll of Washington.

The references to the remaining 26 cases are as follows: E. Fraenkel (4), Ueber Gasphlegmonen, Hamburg u. Leipzig, 1893; Passow (1), Charité-Annalen, 1895, XX., p. 275; Dunham (5), Bulletin of Johns Hopkins Hospital, 1897, VIII, p. 68; Ferguson (1), Trans. Indiana Med. Soc., 1897, p. 339: Erdmann (1), Med. Record, Feb. 5, 1898, p. 205; Le Boutillier (1), Med. Record, March 5, 1898, p. 353; Love and Cary (1), Med. Record, April 8, 1899; Norris (1), Amer. Journ. Med. Sc., 1899, CXVII, p. 195; Hitschmann and Lindenthal (5), Situngsb. d. k. Akad. d. Wiss., Math-Naturw, Cl., Wien, 1899, CVIII, Heft. III, Abth. III, p. 67: Thorndike (2), Boston Med. and Surg. Journal, June 7, 1900, p. 592; Muscatello with Gangitano (3), Riforma med., 1900, II, pp. 508, 519 and 530; Guillemot (1), Compt. rend. Soc. de biol., 1898, 10 s., V, p. 1017. A few other cases in which the gas bacillus was found are not reported with sufficient detail to be available for analysis. It is safe to say that *B. aerog. caps.* has now been found in over 50 cases of gaseous gangrene.

Baltimore, most of the cultures having been studied in my laboratory; of the foreign group of cases all are reported from Germany, Austria, Italy and France, four by E. Fraenkel, one (not absolutely certain) by Passow, five by Hitschmann and Lindenthal, three by Muscatello assisted by Gangitano, and one by Guillemot.[47]

Cases of gaseous phlegmon in which *Bacillus aerogenes capsulatus* was not demonstrated are not included, although many of these presented the same clinical characters and doubtless in some at least the gas bacillus was the active agent. This was true especially of the cases of gangrène foudroyante, usually without satisfactory bacteriological examination, attributed by French writers to Pasteur's *Vibrion septique.* Gertler's[48] eight cases of gaseous phlegmon cannot be utilized for our purposes at all, as they are without any satisfactory bacteriological reports.

A complete analysis of these 46 cases would afford material more than sufficient to occupy this entire address, so that I shall be able to present here only some of the more important points.

Thirty-five of the patients were males, ten females, and of one the sex is not stated. The preponderance of males is to be explained by the fact that most of the cases were due to severe injuries. Robust workmen in the prime of life furnished the largest contingent of cases.

In 80 per cent of these cases one of the extremities was the seat of the emphysematous gangrene, the lower being affected a little over twice as often as the upper extremities. In several instances the emphysema extended from the thigh to the abdominal wall or from the arm to the subcutaneous tissues of the shoulder and chest. There were three examples of primary emphysematous phlegmon of the abdominal wall, of these one following removal of the appendix (Bloodgood), one from an unrecognized strangulated Littré hernia (Martin), and one affecting the deep tissues of the nephrectomy wound (Muscatello). In all of these the infection is believed to have started from the intestine. In one of Dunham's cases there was emphysematous gangrene (originating in a prostatic abscess opening in the buttock) of the scrotum, penis, and anterior abdominal and thoracic subcutaneous tissues. In three instances (Carroll (2), Dobbin (1) reported by Bloodgood) the breast and submammary tissues were the primary seat of the disease, all of these resulting from the infusion of normal salt solution. In one case (Dunham) the gaseous phlegmon appeared at the angle of the lower jaw after incision of a foul submaxillary abscess. In one instance (Welch and Flexner) it started within the pelvis from traumatic rupture of the rectum and extended through the sciatic notch down the thigh.

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[47] Soupault and Guillemot (Bull. et mém. Soc. méd. d. hôp. de Paris, 1900, 3 s., XVII, p. 216) have reported two gaseous abscesses following hypodermic injections of salt solution, and a third which they regard as metastatic, in which they found the gas bacillus (*B. perfringens*), once in pure culture. These cases came to my notice too late to be included in my statistics. All were relatively benign cases.

[48] Ueber Gasphlegmonen. Inaug.-Diss. Halle, 1898.

Of especial interest are three examples of multiple or metastatic emphysematous gangrene: one of the forearm and opposite shoulder, another of the thigh and both shoulders, and still another of one shoulder and the buttocks. In the older literature are similar cases; thus Nélaton observed emphysema not only in the injured leg but also in the opposite, uninjured extremity. In 1897 Leech[49] reported, without adequate bacteriological examination, a case of emphysematous gangrene of the right leg following about three weeks after injury of the right thumb, which became inflamed, there being no evident local cause of the affection of the leg. These cases are to be explained by transportation of the bacilli through the lymphatic or blood current from the primary focus of entrance.

In all but five of the forty-six cases the emphysematous gangrene followed traumatism or a surgical operation. The injuries were as follows: compound fractures, 18; bullet and gunshot wounds, 7; infusion of salt solution, 3; hypodermic injections, 2; ligation of the femoral artery for aneurism, 3; external urethrotomy, 2; traumatic rupture of the rectum, removal of the appendix, prostatic abscess following self-catheterization, operation for strangulated Littré hernia, incision of foul submaxillary abscess, and nephrectomy, each one. Of the five nontraumatic cases the gaseous gangrene followed erysipelas in one, was consecutive to apparently spontaneous gangrene in two, whether diabetic or not is not stated, and was without apparent explanation in two (Fraenkel's case 2 and Passow).

Compound fractures and next bullet and gunshot wounds occupy by far the most prominent place in this list, each of the other various causes being represented only by scattered cases. Those injuries, in which there are much laceration and crushing of tissue, comminution of bone and grinding of dirt, bits of clothing or other foreign bodies into the wound are the ones most likely to be followed by emphysematous gangrene. That, however, severe traumatism is not an essential factor is shown by the five cases (10.9 per cent of the entire number) following hypodermic injections and infusion of normal salt solution. These latter, however, were all of patients whose vital forces were greatly depressed, namely, by Asiatic cholera, typhoid fever, surgical shock, or post partum haemorrhage. There is good reason to believe that the intact tissues of human beings in health possess marked resistance to the gas bacillus.

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[49] Quart. Med. Journ. (Sheffield), 1896-97, V, p. 237.

In the great majority of cases it is evident that the gas bacillus was introduced through the wounded skin from without. In three instances (removal of appendix, strangulated hernia, and traumatic rupture of the rectum) the infection undoubtedly came from the intestine. This was also the probable source of infection in one of Muscatello's cases (gaseous phlegmon in the site of an extirpated kidney). With our present knowledge of the frequent, if not regular, presence of *Bacillus aerogenes capsulatus* in the intestine, there is nothing surprising in this mode of infection. In three of Dunham's cases the infection followed injury of the urethra, and here also the bacilli may have come from the intestine. In one of his cases (gaseous phlegmon at angle of the jaw) it was suggested that the bacilli gained entrance through decayed teeth.

Bloodgood thinks it probable that in one of his cases the gas bacilli were brought by the circulation to the seat of infection. In this case the femoral artery was ligated for traumatic arteriovenous aneurism in the popliteal space. There was no primary infection of the wound, but gangrene of the foot and leg ensued, and on incision gas bubbles were found in the blood of the aneurismal sac and the tissues. With what we know about the entrance of intestinal bacteria into the circulation, there is nothing improbable in Bloodgood's opinion. The clot in an aneurism and tissues robbed of their nutrient supply would offer little or no resistance to the growth of bacilli which might reach them or their immediate neighborhood through the circulation, and the conditions would be anaerobic. It was indeed a case of aneurism in which I first found the gas bacillus, and here the clot was swarming with bacilli. It is interesting to note that three of the cases of emphysematous gangrene in my list followed ligation of the femoral or popliteal artery for aneurism.

Pirogoff[50] distinguished clinically two groups of cases of traumatic emphysematous gangrene. He described under the name "primary mephitic gangrene" cases in which the emphysema appears within two days after the injury, the "local stupor" passing without inflammatory reaction into crepitating gangrene. Here the emphysematous necrosis spreads rapidly, the patient sinks into collapse with an icteroid hue of the skin, small, thready pulse and cold sweats, and death occurs usually within a few days after the onset. This type of the disease corresponds to Maisonneuve's gangrène foudroyante. In the second group, designated by Pirogoff also as "acute gangrenous oedema," there is reaction from the primary "local stupor" of the tissues, the emphysema is preceded and accompanied by local, oedematous or purulent inflammation, is associated with febrile reaction, often appears later after injury and spreads less rapidly, and presents in general a more varied anatomical and clinical picture than the first class of cases.

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[50] *Op. cit.*, p. 1006.

Hitschmann and Lindenthal consider that Pirogoff's first group corresponds especially to cases of pure or predominant infection with the gas bacillus, and his second group to mixed infections. Erdmann is also of the opinion that in the unmixed infections the emphysematous necrosis spreads more rapidly and is more likely to terminate fatally. On the other hand Muscatello and Gangitano, who also divide the cases into two groups—pure infections and mixed infections—hold that the mixed infections are characterized by the rapidly progressive form of emphysematous gangrene, while the pure infections, at least in their early stage, show little tendency to spread beyond the injured tissues. According to the last named authors the gas bacillus is incapable of exerting any pathogenic action upon healthy tissues, but attacks only tissues already altered in their vitality by injury, other pathogenic microorganisms, toxins, or other depressing factors.

The study of our cases has not enabled me to adopt either of the two conflicting opinions just stated. Of the collected forty-six cases, thirty were mixed infections, fourteen were pure, and in two there is no clear statement on this point. The most common associated bacteria were the pyogenic streptococci and staphylococci. Among other forms found occasionally were *Bacillus coli,* proteus, pyocyaneus, tetanus, Sanfelice's *Bacillus pseudo-oedematis maligni,* and uncultivable, often spore-bearing, bacilli. It seems probable that the bacillus of malignant oedema, being a common inhabitant of the soil, must occasionally, like the tetanus bacillus, be present, but it was not isolated from any of these cases. My experience is that if reliance be not placed exclusively upon cultures, but careful microscopical examination be also made, instances of unmixed infection with the gas bacillus are rare. It is by no means always easy to determine whether associated bacteria are exerting pathogenic action or not. Pyogenic cocci may be present without producing pus or marked inflammatory reaction.

We have found rapidly progressive forms of emphysematous gangrene both with pure infections with the gas bacillus and with mixed infections, and on the other hand we have observed with both types of infection cases in which the tendency to spread is much less marked. In all of the cases with much purulent inflammation mixed infection was present, but aside from this feature the division of the cases into pure and mixed infections does not, according to our experience, correspond to different, sharply marked, clinical features. Pirogoff's classification is doubtless of clinical value, but there is every gradation between the two groups.

I cannot agree with Muscatello and Gangitano, whose investigations of emphysematous gangrene are of great value, that the gas bacillus is incapable of attacking healthy tissues. Virulent cultures, even in small doses, can produce rapidly spreading gaseous phlegmons when inoculated into the subcutaneous tissues of susceptible animals, such as guinea pigs and pigeons. In human beings the emphysema may extend very rapidly into the healthy tissues, frequently outstripping in its advance the inflammatory oedema. This may occur in pure, as well as in mixed, infections with the gas bacillus. It is true, as urged by Muscatello and Gangitano, that toxins derived from the bacilli may prepare the tissues for the invasion and action of the advancing bacilli, but the same explanation has been put forward for the spread of other pathogenic bacteria in healthy tissues. In asserting that the gas bacillus may attack healthy tissues, I would not be understood as minimizing the great importance of the various accessory causes of emphysematous gangrene which act by lowering the vitality of the tissues or the general resistance of the patient, or as failing to recognize the marked resistance to infection by the gas bacillus offered by the healthy tissues, a resistance to which I have previously called attention and which I shall have occasion further to emphasize.

Gas may appear in the tissues as early as eight hours after the injury. In a case of gaseous phlegmon following a bullet wound of the knee joint, reported by Welch and Flexner, Dr. Bloodgood recognized gas in the joint and surrounding tissues just twenty hours after the injury.[51] In a case of Dr. Tiffany's, which we have reported, death from emphysematous gangrene, due to pure infection with the gas bacillus, occurred forty-eight hours after a fall causing a compound, communited fracture of the patella with grinding of the underclothing into the wound. There was no other injury of the body. Any one who has seen one of these rapidly fatal cases of spreading, traumatic, emphysematous gangrene will receive an impression which he will never forget.

The anatomical and clinical study of uncomplicated emphysematous gangrene demonstrates that the disease is not, as many formerly supposed, simply an intense variety of ordinary phlegmonous inflammation or cellulitis, is a disease *sui generis*. It may be combined with phlegmonous inflammation, but then some other microorganism, usually the streptococcus, is associated with the gas bacillus.

In typical uncomplicated cases the lesions consist in necrosis of all the tissues, the presence of gas in the interstices, infiltration with the blood, evidences of the mechanical action of the gas, and exudation of a variable amount of bloody serum. The amount of gas varies much in different cases. There may be only a few bubbles or the tissues may be everywhere blown up with gas. The nuclei disappear by karyolysis. The notable thing in most cases is a nearly complete absence of leucocytes and of cellular reaction, although in a few instances, even of pure infection, I have found leucocytes in considerable number, and even purulent foci, but generally at a distance from the primary necrosis. As will be shown later *Bacillus aerogenes capsulatus* in certain situations is capable of setting up purulent inflammation. In one of our cases, reported by Bloodgood, Dr. Cushing found gas bacilli without gas in a small subcutaneous abscess of a stump two months after amputation of the thigh for emphysematous gangrene of the leg, the amputation having been followed by uninterrupted convalescence.

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[51] It is to be regretted that in the cases reported by Prutz (Deutsche Zeitschr. f. Chirurg., 1898, XLVIII, p. 591) as traumatic entrance of air into the knee joint, no bacteriological examination was made.

After death there may be rapid extension of the subcutaneous emphysema, and at autopsies made a few hours after death gas bubbles may be found in the heart, vessels, liver and other organs, but as to this occurrence there is no rule. As I have already explained widespread distribution of gas in the blood and organs in early autopsies indicates entrance of the bacilli into the circulation during life.

As is to be expected from the etiology of many of the cases of emphysematous gangrene, emboli of fat and of bone-marrow cells are common in the pulmonary capillaries, but I do not know that in any case they contributed to the fatal result.

The prognosis of emphysematous gangrene is more favorable today than before the antiseptic period. The disease terminated fatally in 59 per cent of the cases in my list. In the cases observed and treated at The Johns Hopkins Hospital—ten in number—the fatality was 50 per cent, but of these one was a gaseous phlegmon of the pelvis extending to the thigh, resulting from traumatic rupture of the rectum; a second was a gaseous phlegmon of the abdominal wall following removal of the appendix and complicated with diffuse gangrenous peritonitis, and a third case terminated twenty-five days after disappearance of a gaseous phlegmon of the thigh (treated by incisions) a late streptococcus infection, gas and the gas bacillus having disappeared. Of the two remaining fatal cases, in one—emphysematous gangrene following compound fracture of the thigh—amputation was refused by the patient until the fourth day when he was in a state of collapse and died fifteen hours later, and in the other—compound fracture of the skull and both lower extremities—the patient died thirty hours after the accident, having never regained consciousness. When the disease is accessible to surgical treatment, is not complicated by other grave conditions, and is promptly recognized and treated, the prognosis, according to the experience of my surgical colleagues, Professor Halsted and Dr. Bloodgood, is not very unfavorable.

The clinical evidence seems to me to favor the view that, at least in most uncomplicated fatal cases, death is due to toxaemia. Norris was unable to demonstrate the presence of strong toxins in artificial cultures of *Bacillus aerogenes capsulatus,* but, as is well known, the same difficulty is encountered with many other pathogenic bacteria which are believed to produce poisons in the human body. Muscatello is also of the opinion that the constitutional symptoms are attributable to toxaemia.

The suggestion that death may be due to gaseous embolism is not new. Pirogoff and other of the older observers knew that gas bubbles may be found within the heart and vessels very soon after the death of patients from emphysematous gangrene. In the discussion on Langenbeck's paper on traumatic infiltration before the Society of German Military Surgeons in 1870, Senator[52] advocated the idea that death is caused by gas embolism. While the possibility of this occurrence as the cause of death may be admitted, there is at present no proof of this opinion. Gas bubbles are by no means always found in the blood and organs after death from emphysematous gangrene, and the clinical histories of those in whom they are found post mortem do not appear to differ from those where they are absent. In experimental gaseous phlegmons in guinea pigs there is no evidence of the presence of gas bubbles in the circulation during life.

In one of our cases (Mann) death resulted from tetanus, and Verneuil[53] has reported three cases, which without bacteriological examination he calls malignant oedema, where tetanus intervened. There is nothing surprising in this association when one considers that the home of the tetanus bacillus, like that of the gas bacillus, is the soil. The period of incubation for tetanus is longer than for infection with the gas bacillus, the former appearing rarely before the seventh day, and the latter usually within two or three days after the injury.

Prophylactic measures against emphysematous gangrene are sufficiently obvious from the etiological factors which have already been considered. It is important in wounds of the character most frequently followed by this complication to search microscopically and by cultures for the gas bacillus. The examination of cover-slips will usually suffice for a probable diagnosis. I have already cited instances in which this bacillus has been detected before the onset of emphysema and in which there is good reason to think prompt surgical treatment warded off severe infection. The possibility of infection from the intestinal canal, as well as from external sources, is to be borne in mind.

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[52] Deutsche militärärztl. Zeitschr., 1872, I, p. 260.

[53] Semaine méd. 1890, p. 403.

The cases were treated either by free incisions or by amputation or both. The results were better after amputation than after simple incisions. Of the cases of emphysematous gangrene affecting primarily the extremities, the recoveries numbered 68 per cent after amputation, and 33⅓ per cent after incision without amputation. Careful study of individual cases shows that amputation is by no means always necessary. Everything depends upon early recognition of the nature of the infection. Dr. Bloodgood,[54] from a relatively large experience says: "If the infection is recognized early, and the destruction of the soft parts and bone is not extensive, free incisions, with immediate continuous bath treatment, should be tried. If the general symptoms of infection are not immediately relieved, amputation should be done. If, however, the infection is recognized late one should take no risk but amputate at once. . . . . An early diagnosis will probably save life, and from many observations an amputation may not always be necessary."

A similar position as to prognosis and treatment is taken by Muscatello and Gangitano in their valuable paper on gaseous gangrene, in which they also emphasize the value of abundant irrigations with disinfectant and oxidizing solutions. In the service of Dr. Halsted at The Johns Hopkins Hospital the continuous bath treatment has been found useful. Hitschmann and Lindenthal are certainly mistaken in their assertion that incisions are usually without favorable effect, and that early amputations offer the only chance of recovery. As pointed out by Thorndike and others, recovery may follow amputation even when it is impossible to remove the whole of the diseased tissues.

UTERINE INFECTIONS

Knowledge of *Bacillus aerogenes capsulatus* is of not less interest and importance to the obstetrician than to the surgeon. Infection of the puerperal uterus by this microorganism leads to a variety of morbid conditions, some comparatively mild, others of the utmost gravity.

The wide distribution of the gas bacillus in the intestinal canal and the outer world renders as explicable the occasional presence of this bacillus in the female genital tract as that of the colon bacillus in the same situation. Lindenthal[55] found the gas bacillus in the vagina without the presence of gas or other pathogenic effects twice out of six puerperal cases examined. According to the observations of others, Lindenthal's experience would seem to be exceptional. As with so many other pathogenic bacteria the mere presence of the organism upon exposed surfaces does not necessarily signify infection.

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[54] *Op. cit.*, p. 174.

[55] Wiener klin. Wochenschr., 1897, pp. 3 and 35.

I shall consider the uterine infections by the gas bacillus under the headings of emphysema of the foetus, puerperal endometritis, physometra, emphysema of the uterine wall, and puerperal gas-sepsis, although these conditions may be associated with each other.

Emphysema of the Foetus.—The occurrence of emphysema of the dead foetus in utero has been known for centuries,[56] but it was not until 1897, when Dobbin published his paper on "Puerperal Sepsis Due to Infection with *Bacillus aerogenes capsulatus*"[57] that the cause of this condition was determined to be invasion by the gas bacillus. In this case gas and the gas bacillus were both recognized during the life of the patient in the foetus, the placenta and the cavity of the uterus, and after death there was general gas formation throughout the body.

Dr. Dobbin has kindly given me the notes of a second, unpublished case observed by him. He was called to see a woman in difficult labor, upon whom repeated attempts at delivery of a dead foetus had been made. Upon examination he recognized crepitation of the caput succedaneum, while the foetus was still in utero. Upon delivery the foetus was emphysematous with foamy organs. The gas bacillus was found in pure culture in the foetus and mixed with other bacteria in the amniotic fluid. No gas was recognized in the uterus after delivery. The patient made a good recovery without evidence of sepsis.

Menge and Krönig[58] have observed three instances of foetal infection with the gas bacillus, and have brought strong evidence that usually the invasion is through the mouth of the foetus, the bacilli being taken into the lungs or stomach by inspiring or swallowing amniotic fluid. In their cases the bacilli were not found in the part of the umbilical cord attached to the placenta, but in Dobbin's first case this as well as the placenta itself was infected with the gas bacillus. The amniotic fluid within the intact membranes, as is well known, is usually sterile, and only exceptionally becomes infected before rupture of the membranes, so that the infection of the foetus from this source is generally after this rupture. As shown by Menge and Krönig all grades of invasion of the foetus by the gas bacillus occur, so that there may be only a small amount of gas, and this limited to the lungs or the alimentary canal or both. The medico-legal importance of not mistaking for air this gas in the foetal lungs produced by gas bacilli should be emphasized.

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[56] It is usually stated that this condition was known to Celsus, but he does not expressly mention the presence of gas, although this is to be inferred. In the chapter on the extraction of the dead foetus, he says: "Solet etiam evenire, ut is infans humore distendatur, exque eo profluat foedi odoris sanies." Milligan's "Celsus," p. 394, Edinburg, 1831.

[57] Bulletin of The Johns Hopkins Hospital, 1897, VIII, p. 24.

[58] Bakteriologie des weibl. Genitalkanales, Teil II, p. 167, Leipzig, 1897.

As is well known, foetal emphysema is usually unattended with danger to the mother. In two of the five cases due to *Bacillus aerogenes capsulatus* in my list the puerperium was even without rise of temperature, in two there was mild fever, but in Dobbin's first case there was rapidly fatal gas sepsis.

Puerperal Endometritis.—Under the heading "gas sepsis" will be considered cases of acute uterine infection with the gas bacillus, followed by invasion of the blood and organs by this organism. Here it may be mentioned that *Bacillus aerogenes capsulatus* may be present in the uterus, usually in association with other bacteria, in both mild and severe cases of puerperal endometritis without the recognition of gas in the foetus, or the uterine cavity, or wall. An example of such a case is an unpublished one in the service of Dr. J. Whitridge Williams, of which the notes have been furnished me by Dr. Dobbin. A woman with rhachitic pelvis, upon whom delivery by forceps had been attempted before admission to the Johns Hopkins Hospital, was there delivered by craniotomy. There was no gas in the foetus. Forty-eight hours later *Bacillus aerogenes capsulatus,* together with streptococci, was found in the uterine lochia. No gas was detected. The patient developed fever, but recovered.

In two cases, one reported by Dobbin[59] and the other by Blumer,[60] the gas bacillus, although not identified with absolute certainty, was probably present in the puerperal uterus in association with the typhoid bacillus.

Physometra.—Distension of the uterine cavity with gas (physometra or tympany of the uterus) was present in Dobbin's first case, already cited, and is often associated with emphysema of the dead foetus, but may occur without the latter, and even in the nonpregnant uterus. This curious condition was formerly ascribed to entrance of air or to ordinary putrefaction,[61] but it is now known to be the result of the activity of gas-producing bacilli.

Since the observations of Lindenthal,[62] reported in 1898, it cannot be doubted that *Bacillus aerogenes capsulatus* is the chief cause of physometra. He found this bacillus in five cases during life, and reproduced the condition experimentally in guinea pigs. He is justly skeptical of the correctness of the previous reports of Gebhard[63] concerning the agency of the colon bacillus in generating gas within the uterus. We know from Theobald Smith's investigations that the colon bacillus can produce gas only from carbohydrates, whereas it is the most distinctive biological attribute of our gas bacillus that it can produce gas from proteids. Until it has been shown that the amniotic fluid and uterine contents may, under any conditions, contain enough carbohydrate to explain the development of gas by the colon bacillus, there is every reason to question the claims for this bacillus as a cause of tympany of the uterus.[64]

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[59] Am. Journ. Obstetrics, 1898, XXXVIII, p. 185.

[60] *Ibid.*, 1899, XXXIX, p. 42.

[61] The older hypotheses and records on physometra and emphysema of the foetus are fully presented by Staude, Zeitschr. f. Geb. u. Gynäk., 1878, III, p. 191.

[62] Monatsschr. f. Geb. u. Gynäk, 1898, VII, p. 269.

[63] Zeitschr. f. Geb. u. Gynäk., 1893, XXVI, p. 480, and 1897, XXXVII, p. 132.

My list of cases contains ten instances of physometra in which *Bacillus aerogenes capsulatus* was demonstrated. Although, as some of our cases show, this condition may be associated with invasion of the bacilli into the wall of the uterus, and by acute gas sepsis, these occurrences are exceptional, and the prognosis is in general a favorable one. Most of the cases furnish a good illustration of the resistance of living human tissues to the action of the gas bacillus. Doubtless, in these cases, the bacilli grow simply in the amniotic fluid after rupture of the membranes, and in the dead foetus, these offering no vital resistance, whereas we must suppose that the intact uterine wall offers ordinarily an effective resistance to the invasion and multiplication of the gas bacillus. That occasionally the bacilli may find other dead material in the uterus, as in sloughing myomata and cancers, is evident from the histories of some cases of physometra.

Emphysema of the Uterine Wall.—Far graver in significance is septic emphysema of the uterine wall of which Halban[65] has recently reported an interesting case due to *Bacillus aerogenes capsulatus.* Graham, Steward and Baldwin and P. Ernst were the first to demonstrate this bacillus in this condition, their papers, to which reference has already been made, appearing simultaneously in August, 1893. Eleven instances of emphysema of the wall of the uterus, all puerperal, have been reported, of which five were recognized during life. In all but Halban's case there was also physometra, and this would seem to be a necessary accompaniment unless the cervical canal is open, so as to permit the escape of the gas from the uterine cavity. All of the cases were fatal, and in most gas was found at autopsy in the blood and internal organs.

Subperitoneal emphysema is a condition which has been observed after rupture of the uterus, Dischler[66] having collected reports of 14 cases. In most instances this has been attributed to entrance of air, but I think that it is safe to predict that the gas bacillus will be found in similar cases in the future, if proper methods for its detection are employed. I know, however, of no instance of this condition in which it has been looked for.

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[64] Halban (Monatsschr. f. Geb. u. Gynäk., 1900, XI, p. 102), states that he has found lactose once in the amniotic fluid of a normal puerpera. Further studies of this subject are needed.

[65] Monatsschr. f. Geb. u. Gynäk., 1900, XI, p. 88.

[66] Arch. f. Gynäk., 1898, LVI, p. 199.

Puerperal Gas Sepsis.—I have adopted from Halban the term "gas sepsis" as a convenient one, although perhaps open to criticism, to designate the important group of fatal puerperal cases in which gas bubbles are found at early autopsies in the heart and vessels and often also in the organs and tissues under conditions where we must suppose that gas bacilli and possibly gas have passed from the uterus into the circulation during life.[67] Here in my opinion belong most of the cases which have been reported as deaths due to the entrance of air into the uterine veins. This opinion, which I expressed in my first communications on the gas bacillus in 1891 and 1892, received prompt confirmation in the papers of Graham, Steward and Baldwin and P. Ernst in August, 1893, and has since been strengthened by other similar observations. Cases described by Wendeler[68] as sepsis acutissima belong also in the same category.

My list includes thirteen puerperal cases in which gas bubbles and the gas bacillus were found at autopsy in the blood or organs, but in only seven of these does it seem to me conclusive or extremely probable that the infection occurred during life.[69]

The most remarkable of these cases is that reported by Graham, Steward and Baldwin of a woman, upon whom abortion had been recently produced, who during the four hours immediately preceding death became emphysematous over nearly the whole body. At the autopsy gas and gas bacilli were found everywhere throughout the body. In Dalton and Bremer's[70] case, also one of criminal abortion, an emphysematous swelling of the arm and pectoral region was likewise recognized during life. These cases are of importance as demonstrating the invasion of the body by the gas bacillus from the uterus while the blood is still circulating. In Halban's and Dobbin's cases gas was also recognized during life within the wall or cavity of the uterus.

In the majority of cases of puerperal gas sepsis there has been some operative interference preceding infection, such as criminal abortion, forced delivery for placenta praevia or other causes, or the manipulations of an unskilled midwife. The fulminating character of the infection, death being sometimes very sudden, is a notable feature of many of the cases.

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[67] Gas sepsis in this sense occurs also in other than uterine infections with the gas bacillus.

[68] Monatsschr. f. Geb. u. Gynäk., 1896, IV, p. 581.

[69] These are the cases reported by Graham, Steward and Baldwin, P. Ernst, Krönig and Menge, Dobbin, Cesaris-Demel, F. C. Wood, (Med. Record, Apr. 15, 1899), and Halban, to whose papers references have already been given.

[70] Am. Journ. Med. Sc. 1888, XCV, p. 594. This infection, although attributed to the bacillus of malignant oedema, was probably due to the gas bacillus.

In a case of attempted criminal abortion reported by Perkins[71] the patient, according to the statement of the physician in whose office death occurred, died suddenly, and at the autopsy, twelve hours later, in cold weather, gas was found in the vena cava, heart, and other vessels, with evidences of injury to the pregnant uterus. The case was reported by Perkins as one of death from air embolism, and certainly with as much and even more plausibility than most cases thus reported. After the publication, Dr. Perkins, upon the request of Dr. Dobbin, was so good as to send the uterus, well preserved in alcohol, to my laboratory where Dr. Dobbin demonstrated in the uterine vessels and tissue bacilli morphologically and in staining reaction identical with *Bacillus aerogenes capsulatus*.[72]

I would not be understood to deny the possibility of the occurrence of fatal air embolism from the uterus. A very few of the reported cases are difficult to interpret upon any other supposition, but I do claim that the foundations of this doctrine have been seriously shaken by our discovery and investigations of the gas bacillus, and that no case, however plausible, can be considered as positively proven without a satisfactory bacteriological examination. The limitation of gas to the right heart and adjacent vessels may occur from invasion by the gas bacillus and is not, as is often represented, peculiar to air embolism. Did we not know how long it takes new knowledge, especially that originating in this country, to penetrate throughout the medical world, it would be amazing that cases should still continue to be reported, as they are,[73] of deaths ascribed to air embolism without any bacteriological examination or even any reference to the possibility of any other explanation. I am not aware that in any instance of alleged air embolism, a bacteriological examination has been made, which would exclude the presence of gas-forming bacteria.

Whether, as suggested by Staude, in any of the cases with gas within the uterine cavity, death is attributable to gaseous embolism, is, I think, an open question. It is possible, although I know of no proof of it, that in some of the cases of sudden death during or immediately after some manipulation or operation on the pregnant uterus and attributed to air embolism, gas, generated by bacteria, may have existed under pressure within the uterine cavity and have entered wounded veins in sufficient amount and so suddenly as to have caused death.

To what extent the free gas found in the blood vessels, heart and internal organs, even very soon after death from what has been described as puerperal gas sepsis, in there during life, is a question difficult to answer. I have already considered this subject, and in this connection shall again emphasize the importance of caution in interpreting the presence of gas in these situations as a vital phenomenon, although there is evidence that it may be such.

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[71] Boston Med. and Surg. Jour., 1897, CXXXVI, p. 154.

[72] Dobbin has reported his examination with further notes of this case in the Monatsschr. f. Geb. u. Gynäk., 1897, VI, p. 375.

[73] Zorn (Münch. med. Woch., 1898, p. 567) may be cited as an example.

INFECTIONS OF THE URINARY TRACT

There is evidence that the urinary tract may not only be a portal of entrance for the gas bacillus into the circulation or adjacent tissues but also be itself the seat of infection by this organism. Unfortunately for the decisive interpretation of many of these cases, *Bacillus aerogenes capsulatus* has, so far as I am aware, hitherto been found in the urinary tract only after death, although in some instances so soon thereafter and under such conditions that its presence during life cannot be doubted.

I have already called attention to instances of emphysematous gangrene following external urethrotomy and other operations on the urinary passages.

Among the cases of general invasion of the blood and organs (foamy organs) by the gas bacillus observed by myself and others are several in which the portal of entry was the urethra, bladder or other part of the urinary tract.[74] In a case of urethral stricture with cystitis, for which perineal section has been done, reported by Welch and Flexner, gas bacilli were found *three-quarters of an hour after death,* in large numbers in the bladder, ureters and renal pelvis and a few gas bubbles and gas bacilli were already present in the blood of the right ventricle. In Howard's case of meningitis, caused by the gas bacillus, to be cited subsequently, he considers that the portal of entry was the urinary tract.

Gas, generated by *Bacillus aerogenes capsulatus,* has been found in the urinary passages in six cases which have come to my notice.[75] The gas may be either free in the cavity of the bladder, ureters or renal pelvis, or contained within submucous blebs, or in both situations.

Welch and Flexner [76] have reported an instance of pneumaturia in a diabetic man in whom six hours after death, without trace of cadaveric decomposition, the urinary bladder was found filled with frothy urine containing *Bacillus aerogenes capsulatus* in pure culture. This case indicates that the colon bacillus and *Bacillus lactis aerogenes* are not the sole causes of pneumaturia in diabetics. Dr. Flexner has given me notes of a second case of pneumaturia upon which he made the autopsy at the University Hospital, Philadelphia. This was of a patient with chronic cardiac disease, who had been catheterized twenty-eight hours before admission, and who died thirty hours after admission. At the autopsy, made one hour and a half after death, about 60 c. c. of frothy urine were found in the bladder, from which *Bacillus aerogenes capsulatus,* together with *Staphylococcus aureus* and *Streptococcus pyogenes* (no colon bacilli), was cultivated. Neither gas nor gas bacilli were found elsewhere in the body. The mucous membrane of the bladder was oedematous. There can be little doubt that in this case the gas bacillus was introduced by the catheter into the bladder.

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[74] Such cases are reported by Welch and Flexner, Goebel, Dunham, and Howard in papers already cited.

[75] These do not include some instances of very extensive post mortem emphysema of the organs with gas everywhere throughout the body.

[76] Case XIII of our list in Journal of Experimental Medicine, 1896.

In one of Welch and Flexner's cases (Case XVIII) of hypertrophied prostate with pyoureter and pyonephrosis the renal pelves and ureters were found at autopsy distended with gas and containing pus mixed with bubbles of gas. Small gas cysts were present in the mucous membrane of the renal pelves. Neither gas nor gas bacilli were present outside of the urinary organs. In this case cocci and colon bacilli were associated with the gas bacillus.

In a case of Dr. Kelly's, of which the full records have been given me by Dr. Miller,[77] there was pneumaturia demonstrated by cystoscopic examination and ureteral catheterization to come exclusively from the inflamed left renal pelvis and ureter. Among cocci and other bacteria were found on cover-slips bacilli morphologically resembling *Bacillus aerogenes capsulatus,* but unfortunately no anaerobic cultures were made. None of the bacteria which grew aerobically produced gas in lactose agar. This case is interesting as demonstrating that the gas may come exclusively from one renal pelvis and ureter, but the microorganism producing the gas was not satisfactorily demonstrated. It seems certain that it was an anaérobic organism, and from the microscopical appearances may have been the gas bacillus.

The subject of submucous gas cysts will be considered subsequently; but here it may be mentioned that besides the gas cysts in the renal pelves noted by Welch and Flexner in the case already cited, Goebel found gas blebs, containing in pure culture *Bacillus aerogenes capsulatus,* beneath the mucous membrane of the urinary bladder, without gas elsewhere in the body, and Dunham has reported a like condition of the bladder in a case of emphysematous gangrene with general invasion of the blood and organs by the gas bacillus.

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[77] This case is briefly reported by Kelly and MacCallum, Journ. Amer. Med. Assoc., 1898, XXXI, p. 376, whose paper may be consulted for the full literature of pneumaturia.

Bacteria have been found in emphysema of the bladder (cystitis emphysematosa) also by Eisenlohr,[78] Camargo[79] and Kedrowsky;[80] but it is impossible, from the authors' descriptions, to identify their bacteria. Kedrowsky considers the bacillus isolated by him as allied to *Bacillus aerogenes capsulatus,* but less sensitive to oxygen. Perhaps it was Sanfelice's *Bacillus pseudo-oedematis maligni,* to which I have already referred, but Kedrowsky's description of his cultures hardly inspires confidence in their purity.

Welch and Flexner, and Howard have reported finding the gas bacillus, in association with other bacteria, in inflammatory lesions of the bladder, renal pelvis and kidneys, without the detection of gas. Howard considers that in one of his cases the bacillus was concerned in the etiology of suppurative lesions of the kidneys, but in this case the colon bacillus and *Streptococcus pyogenes* were also present.

Infections Derived from the Gastro-Intestinal Canal

Mention has already been made of the frequent, if not constant, presence of *Bacillus aerogenes capsulatus* in the intestinal canal, of gaseous phlegmons originating from this source, and of the readiness with which intestinal bacteria may gain access to the genito-urinary tract.

The intestine is by far the most common source of the gas bacilli found together with gas bubbles in the blood and organs at autopsies. This invasion may occur either with or without definite intestinal lesions, and is probably in the majority of cases an agonal or post mortem event. The mode of distribution and spread of the bacilli in these cases has been well described by Howard,[81] whose experience has been exceptionally large with this class of affections. Especially demonstrative of invasion of gas bacilli from the intestine, usually post mortem, is the occurrence of gas bubbles limited to the neighborhood of the intestine, as in the intestinal wall, within the portal or mesenteric veins or lymphatics, in the subperitoneal tissue, mesentery and omenta, around the pancreas, in the mesenteric glands, and especially in the loose tissue near the gall-bladder and in the porta of the liver, without gas in more remote situations. I have seen examples of each of these occurrences in very early autopsies without ordinary cadaveric decompositions.

Local Gastro-Intestinal Lesions.—Interstitial emphysema of the gastro-intestinal wall will be considered subsequently.

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[78] Ziegler's Beitr., 1888, III, p. 101.

[79] Thèse de Genève, 1891.

[80] Centralbl. f. allg. Path. u. path. Anat, 1898, IX, p. 817.

[81] *Op. cit.*, p. 491.

Howard has described several cases with larger or smaller areas of superficial necrosis of the mucous membrane of the stomach and intestine, in which gas bacilli were present in large numbers. These areas, which may occur either with or without gas cysts, are found most frequently beneath the folds of the valvulae conniventes and are characterized by absence of nuclear staining and disintegration of the cells and tissue, usually without marked inflammatory reaction.

Bacillus aerogenes capsulatus may undoubtedly be a cause of meteorism. Instances of this are reported by Welch and Flexner and by Howard.

Pneumoperitonitis With and Without Perforation.—My records include thirteen cases of diffuse peritonitis in the exudate of which *Bacillus aerogenes capsulatus* was found. Eleven of these were in autopsies made either by Dr. Flexner or myself, seven having already been published by us in 1896. The remaining two cases (both being perforations of gastric ulcers) have been reported by Page,[82] and by Pratt and Fulton.[83] It was the observation of these cases which first called my attention to the frequent presence of the gas bacillus in the intestine.

Ten of the cases were perforative and three were nonperforative. Of the former four were the result of perforation of typhoid ulcers, four of gastric ulcers,[84] one of strangulated, gangrenous intestine and one of a cancerous ulcer of the duodenum. In the last case (autopsy fourteen hours after death) the exudate was serofibrinous and the gas bacillus was found in pure culture,[85] and abundantly in the peritoneal cavity and was absent from other organs and the blood. In the other perforative cases the gas bacillus was mixed with other bacteria, although in some instances it predominated. In all of the cases the abdomen was greatly distended with gas and usually there was great tympanites.

I attach especial importance to the case fully reported by Welch and Flexner,[86] in which we brought conclusive evidence of the occurrence of pneumoperitonitis without perforation, the first of the kind on record in which similar proof was obtained. Since our publication a similar case has been observed by Dr. Flexner in Manila, who has kindly furnished me the notes.

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[82] Canada Lancet, May, 1900.

[83] Boston Med. and Surg. Journ., June 7, 1900, p. 599.

[84] Welch and Flexner have reported an instance of peritonitis from perforated gastric ulcer in a rabbit, in which the gas bacillus was found.

[85] The purity of the culture in this case is explicable by the results of Cushing and Livingood's interesting bacteriological and experimental studies of the duodenal flora published in Contributions to the Science of Medicine, dedicated by his pupils to William Henry Welch on the 25th Anniversary of his Doctorate, Baltimore, 1900, p. 543.

[86] *Op. cit.*, p. 36.

At the autopsy, twelve hours after death, there was found haemorrhagic infarction of the lower part of the ileum and adjacent part of the large intestine, caused by the passage of this part of the intestine through a hole in the mesentery. The peritoneal cavity was greatly distended with gas which burnt with a pale, blue flame. There was a large amount of frothy, bloody serum in the peritoneal cavity together with a fibrinous exudate. The most careful examination showed no perforation. *Bacillus aerogenes capsulatus* was obtained in pure culture and abundantly from the peritoneal fluid. Gas was absent from the blood and other organs.

We have found the gas bacillus, mixed with other bacteria, twice in circumscribed, gas-containing, intraperitoneal abscesses resulting from perforation of the appendix vermiformis.

Hepatic and Biliary Infections.—The development of gas in the liver is so striking a phenomenon in most autopsies where the gas bacillus and free gas are found in the blood and organs that P. Ernst used the term "Schaumleber" for the title of his article on the gas bacillus, published a year after the paper by Welch and Nuttall, in which we first directed attention to the subject of foamy organs and the gas bacillus.[87] I have already considered the general subject of gas bubbles in the blood and organs, and wish here to call attention especially to infections of the gall-bladder and biliary passages by *Bacillus aerogenes capsulatus.*

In cases of foamy liver gas may be found in the bile-ducts and gall-bladder, but my experience is that, when the gas bacilli reach the liver through the blood vessels, the appearance of gas in these situations is a rather late occurrence and met chiefly in advanced cases. In contrast to these cases are the observations of gas in the biliary passages, associated sometimes with definite lesions of the bile-ducts and liver, where the evidence is that the gas bacilli entered from the intestine directly into these passages. Two such cases have been reported by Howard,[88] and I have observed two cases.

Pratt and Fulton[89] report a remarkable case of cancer of the common bile-duct and pancreas in which cholecystotomy was performed, the opening in the gall-bladder being stitched to the abdominal walls. At the autopsy the liver was found studded with minute abscesses with greenish translucent walls. In cover slips, sections and cultures *Bacillus aerogenes capsulatus* was found in pure culture in these small biliary abscesses, but there was no gas in the abscesses, the liver, blood, or other organs. A somewhat similar case, in which cholecystenterostomy for gall stones had been performed, was previously reported by Nicholls[90] from Adami's laboratory. Here also multiple miliary abscesses containing the gas bacillus were found in the liver, but in this case gas was present in the liver, as well as in the blood and other organs, the autopsy being six hours after death. Larkin[91] has likewise reported a case of haemorrhagic pancreatitis with fat necroses and small, multiple hepatic abscesses with gas holes in the liver, from which *Bacillus aerogenes capsulatus* was isolated. The autopsy was eight hours after death.

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[87] Heydenreich's on "Emphysem der Leber" (Centralbl. f. Bakter., 1897, XXI, p. 305) may be mentioned as a curiosity. He had never heard of the gas bacillus or of any other investigation of the subject later than 1872. In contrast to this is the interesting article of P. Bernhardt on pneumathaemia and foamy organs with full consideration of the recent literature. Deutsche med. Woch., 1900, p. 83.

[88] *Op. cit.*, pp. 475 and 476.

[89] Boston Med. and Surg. Journ., June 7, 1900, p. 599.

Hintze[92] has recorded a post mortem observation of gas in the inflamed bile ducts with cholelithiasis. He cultivated only the colon bacillus, but it does not appear that he made anaerobic cultures.

From the foregoing cases it is to be inferred that the gas bacillus may invade the bile-ducts and gall-bladder from the intestine, and sometimes during life, and that it may produce not only gas but also necroses and purulent inflammation. The presence of gall stones, cancer of the bile-ducts and operations on the gall-bladder appear to favor this mode of infection.

Rist[93] found the gas bacillus (*Bacillus perfringens*), without gas or other lesions attributed to it, in an extirpated gall-bladder containing a calculus and clear viscid fluid.

INTERSTITIAL EMPHYSEMA OF THE GASTRO-INTESTINAL, GENITO-URINARY AND BILIARY TRACTS

One of the most interesting lesions produced by *Bacillus aerogenes capsulatus* is the formation of submucous or subserous gas cysts or blebs, of which the earliest examples attributed to this organism were reported by P. Ernst, Goebel, and Welch and Flexner. They are sufficiently common to have been observed by nearly all investigators who have had much experience with the gas bacillus in human beings. My list of cases includes twenty-five instances of this condition, of which five were of the stomach, eleven of the intestine, far oftener of the small than of the large intestine, five of the gall-bladder and bile-ducts, three of the urinary bladder, one of the renal pelvis, and one of the vagina. The majority of the cases were observed by Flexner, Howard, and myself, and in all the gas bacillus was demonstrated. The cases of emphysema of the vagina was reported by Lindenthal.[94]

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[90] Brit. Med. Journ, 1897, II, p. 1844.

[91] Med. Record, 1898, LIII, p. 354.

[92] Münch. med. Woch., 1895, XLIII, p. 209.

[93] Thèse, Paris, 1898.

[94] Wiener klin. Woch., 1897, pp. 3 and 35.

These gas cysts vary in size from microscopic dimensions to large blebs. They are the most common in the submucous coat, but may be present in the mucous membrane, the muscular coat or beneath the serous covering; in fact in any part of the membranous wall. They may be few or in enormous numbers, in groups or scattered. In one of our cases the whole small intestine from the duodenum to the ileocoecal valve was studded with small gas cysts. Gas cysts of the same general character may be found in the mesentery and omenta.

These gas cysts are, in their inception, simply such gas holes as we are already familiar with in the liver and other organs in cases of local or general invasion with the gas bacillus. They indicate a foamy or emphysematous condition of the walls of the stomach, intestine, gall-bladder, bile-ducts, urinary bladder and vagina, due to the invasion into these parts of the gas bacillus.

The condition of the walls of the blebs and of the surrounding tissues varies. Some of the cavities are round and their walls smooth, others are irregular in shape and have ragged walls. They may correspond to dilated lymphatics, but more frequently they do not represent dilatation of any preformed channels. There may be communication between adjacent cavities, but oftener the cysts are distinct from each other. The tissue in the immediate neighborhood of the cavities may present no alteration not explicable by the mechanical pressure of the gas, or it may show necrosis in varying degree and extent. Inflammatory changes or cellular reaction, which could be reasonably referred to the presence of the cysts or of the gas bacilli causing them, were not noted. Sometimes a little coagulated, homogeneous or granular material is present in sections of the cysts in hardened tissues, as indeed may often be found in gas holes in the organs.

In sections stained by Gram there is usually no difficulty in demonstrating the relation of the gas bacilli to the cysts. The bacilli may be in masses in the walls of the cysts, but sometimes they are not more numerous there than in the tissue at a distance from the gas blebs, and occasionally it requires some searching to find them. By anaerobic methods the gas bacillus can be cultivated from the cysts or the adjacent tissue.

All of the instances of submucous and subserous gas cysts in my list were observed post mortem. In the larger number of cases gas bacilli and gas bubbles were more or less widely distributed in the blood and organs, but without evidences of ordinary post mortem decomposition There are, however, several cases in which these gas cysts, in various situations, were the sole manifestations of the presence of the gas bacillus in the body. Howard has shown that if careful, systematic search is made for areas of necrosis and minute gas cysts due to *Bacillus aerogenes capsulatus* in the stomach and intestine, these lesions can be found much more frequently than has been supposed.

It is, with our present information, a difficult matter to say in how many of these cases the emphysematous state existed before death. It is certain that, at least in the great majority of cases in my list, the emphysema was not the result of ordinary post mortem decomposition. In a large number of the cases the autopsy was made within a few hours after death. Howard, in one of his cases of gas cysts, limited to the intestine, made the autopsy one hour after death. I have already emphasized the importance of great caution in interpreting as vital processes the various gaseous conditions of parts and organs not open to inspection during life, even when autopsies are made soon after death and there is entire absence of putrefaction, and I can only repeat this caution here. Mere absence of nuclear staining around gas holes, I do not regard as proof of their origin during life.

It would lead altogether too far to enter here into discussion of the general subject of submucous gas cysts, concerning which there is a large literature, the principal references to which will be found in the articles of Eisenlohr,[95] Camargo,[96] Winands,[97] Dupraz[98] and Lindenthal.[99] It would appear from a study of the records of the subject that anatomically different conditions have been described under the designation "gas cysts" (cystides aëriferae). Some have been apparently of long standing and show chronic inflammatory changes in their walls and surrounding tissues. The most voluminous records relate to the gas cysts of the vagina (kolpohyperplasia cystica of Winckel, emphysema vaginae of Eppinger).

At present we have no warrant to identify the more chronic gas cysts with thickened walls with the more acute emphysematous condition which I have described as referable to *Bacillus aerogenes capsulatus*. Nevertheless, the former are probably of bacterial origin also, although I am unable to accept the bacteriological findings of Eisenlohr, Camargo, Orlandi and Dupraz as at all convincing; for they made no use of anaerobic methods of cultivation. It is quite possible that *Bacillus aerogenes capsulatus* is concerned also in the etiology of the gas cysts of long standing; certainly it is important to apply hereafter anaerobic cultural methods to their study. Lindenthal has no hesitation in identifying the emphysema of the vagina observed by him post mortem, and from which, in a single case, he cultivated what he chooses to call *Bacillus emphysematis vaginae* (in reality our *Bacillus aerogenes*

[95] Ziegler's Beiträge, 1888, III, p. 101.

[96] Thèse de Genève, 1891.

[97] Ziegler's Beiträge, 1895, XVII, p. 38.

[98] Arch. de méd. expér., 1897, IX, p. 282.

[99] Wiener klin. Wochenschr., 1897, pp. 3 and 35.

capsulatus), with Winckel's kolpohyperplasia cystica; but, while he may be correct, further observations are needed before this anatomical and etiological identification can be accepted.

The only instance in which gas cysts of the human intestine have been recognized during the life of the patient is reported by Hahn[100] who found them in large number upon opening the abdomen. It is to be regretted that no thorough bacteriological examination of this case was made.

In my original communication on the gas bacillus I ventured the surmise that the bacillus found by E. Fraenkel[101] in hardened sections from a case of gastritis emphysematosa might be identical with *Bacillus aerogenes capsulatus,* and this suspicion has gained in probability by Goebel's statements, based upon a reexamination of the sections, his work having been done under Fraenkel's supervision.

Notwithstanding an effort at compression, so much space has been occupied in the presentation of the foregoing subdivisions of my subject, which in less than a decade has grown to considerable magnitude, that I shall only summarize very briefly a few remaining topics, although all deserve fuller treatment.

Pulmonary and Pleural Infections.—To the two instances of invasion of the lungs by the gas bacillus, reported by Welch and Flexner, I can add the cultivation of this organism from a gangrenous lung by Dr. Flexner in my laboratory. It has been found also by Guillemot[102] and Rist[103] in pulmonary gangrene.

Of much importance is Levy's[104] demonstration of *Bacillus aerogenes capsulatus* as a cause of pneumothorax without perforation. This case, and those of pneumoperitonitis without perforation, to which I have already referred, have settled affirmatively the long standing controversy concerning the possibility of the generation of gas within closed serous sacs during life. Nicholl's[105] has reported an instance of pneumothorax and pneumopericardium without perforation, in which the gas bacillus was probably present. Rendu and Rist[106] also isolated the gas bacillus (*Bacillus perfringens*) in a case of putrid pleurisy with gas. May and Gebhart[107] and

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[100] Deutsche med. Woch., 1899, p. 657.

[101] Virchow's Archiv., 1889, CXVIII, p. 526.

[102] Thèse, Paris, 1899.

[103] Thèse, Paris, 1898.

[104] Arch. f. exp. Path. u. Pharm., 1895, XXXV, p. 335.

[105] *Loc. cit.*

[106] Bull. et mém. Soc. méd. d. hôp. de Paris, 1899, 3 s., XVI, p. 133.

[107] Deutsches Arch. f. klin. med., 1898, LXI, p. 323.

Finley[108] attribute their two cases of pneumothorax without perforation to the colon bacillus; but in neither were anaerobic cultures made. It is important that in all gaseous affections anaerobes should be searched for.

Gas Bacillus in the Blood During Life.—Extremely interesting is the demonstration by Gwyn,[109] both by cover slip specimens and by cultures, on repeated examinations, of *Bacillus aerogenes capsulatus* during life in the blood of a patient, in The Johns Hopkins Hospital, with chorea insaniens and acute endocarditis. I had the opportunity of examining his cultures, which were entirely typical. The patient died, but unfortunately no autopsy could be obtained. There was no evidence during life of free gas in the blood or tissues. The case is of importance as demonstrating that gas bacilli may be in the circulating blood for days in sufficient number to be demonstrable, both microscopically and culturally, without evidence of free gas. I have already expressed my suspicion that Achalme's anaerobic bacillus, which has likewise been cultivated from the blood, as well as from the tissues, of several cases of acute articular rheumatism, is identical with *Bacillus aerogenes capsulatus.*

Presence of the Gas Bacillus Without Gas.—In this connection I may say that the gas bacillus may be present and even multiplying within the human body without the production of gas. Certain organs, above all the liver, offer much more favorable pabulum for the generation of gas than do others; but even in the liver the bacilli may be present without gas. I have found gas bacilli in small clumps within the spleen and kidney, surrounded by areas of necrosis, without recognizable gas. I have already cited the demonstrations by Cushing and by Pratt and Fulton of the gas bacillus in small abscesses without gas, the observation of the latter being particularly complete and satisfactory. Rist[110] has cultivated the gas bacillus (*Bacillus perfringens*) from cases of fetid otorrhoea and of mastoid abscess. He does not mention the presence of gas in these cases.

Dr. Harris, in my laboratory, isolated the gas bacillus in pure culture from an abscess containing blood and pus in the neck of a dog, following an operation on the jugular vein. There was no gas in the abscess. Aerobic cultures were entirely negative. These observations, however, are not the only ones demonstrating that the gas bacillus may, under certain circumstances and in certain situations, manifest pyogenic capacity.

Meningitis. Pyogenic Capacity of Gas Bacillus.—Howard[111] in April, 1899, reported a case of acute fibrinopurulent meningitis, following operation for urethro-perineal fistula. *Bacillus aerogenes capsulatus* was found in pure culture in the meningeal exudate. Hitschmann and Lindenthal[112] have recorded another convincing example of acute cerebral meningitis, following fracture of the occipital bone, in which the gas bacillus was found unmixed with other bacteria. These authors call attention to the pyogenic power of the gas bacillus when it attacks the meninges, but, as already shown, the same power may be manifested elsewhere.

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[108] Philadelphia Monthly Med. Journ., 1899, I, p. 569.

[109] Bulletin of The Johns Hopkins Hospital, 1899, X, p. 134.

[110] Thèse, Paris, 1898.

[111] Bulletin of The Johns Hopkins Hospital, 1899, X, p. 66.

Cavities in the Brain.—Finally I would call attention to Reuling and Herring's[113] and Howard's studies of cavities in the brain produced by *Bacillus aerogenes capsulatus* and to the light which their observations shed upon certain obscure examples of cerebral cavities previously reported.

In this excursion into pneumatopathology I have invited you to the survey of fields comparatively new and little trodden. I can only hope that our journey has been not without some interest and some profit to you. To me the opportunity to present before such a body as The Massachusetts Medical Society the results of these investigations is one which I highly appreciate.

I desire in closing to pay my tribute of respect and to call to your grateful remembrance the founder of this lectureship, Dr. George Cheyne Shattuck. That the subject of this address would not have been without interest to him may be inferred from the title of his first Bolyston Prize dissertation on the theme propounded in 1806 and entitled: "The difference between mortification produced by an external cause and that which is produced by a constitutional defect, the diagnosticks and the proper mode of treatment of each."[114] By his noble character, professional services and liberality Dr. Shattuck deserved well of the medical profession of his city and state and indeed of the whole country, and his memory is worthily perpetuated not only by the foundation of this lectureship but also by the endowment of the chair of pathological anatomy in the Harvard Medical School.

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[112] *Op. cit.*

[113] Bulletin of The Johns Hopkins Hospital, 1899, X, p. 62.

[114] Published in Boston in 1808.

DISTRIBUTION OF *BACILLUS AEROGENES CAPSULATUS* (*BACILLUS WELCHII*, MIGULA)[1]

There can be no question but that the bacillus discovered by Welch in 1891, and fully described by Welch and Nuttall in the following year, is identical with Fraenkel's *B. phlegmones emphysematosae,* with Veillon and Zuber's *B. perfringens,* and with Schattenfroh and Grassberger's *Granulobacillus saccharobutyricus immobilis liquefaciens* described in 1900. Klein's description of his *B. enteritidis sporogenes* is not reconcilable with that of any single species. His cultures were a mixture of *B. aerogenes capsulatus* and an anaerobic peptonizing bacillus.

It has already been demonstrated by Welch, by Howard and by Hitschmann and Lindenthal that *B. aerogenes capsulatus* is a widely distributed organism, its natural habitats being especially the intestinal canal of man and other animals, and the soil. Dr. Hirshberg has, during the past summer, made a systematic study of the distribution of this bacillus in various situations. Various methods were employed for its isolation, one of the most useful being the inoculation into the circulation of rabbits, which were then killed according to the procedure described by Welch and Nuttall. In each instance the bacillus, if found, was isolated in pure culture, and identified by its characteristic properties.

B. aerogenes capsulatus was found by Hirshberg regularly in the faeces of man (being isolated from all parts of the intestinal canal), of swine, of dogs and of cats and was found with varying frequency, as a rule, in 50 to 80 per cent of the animals examined, in the faeces of rabbits, guinea pigs, mice, rats, chickens, pigeons and cows. It was likewise obtained from the excrement of flies hovering around the bodies of infected animals or human cadavers. It was isolated constantly from garden earth, rarely from street dust. It was detected four times out of eighteen in examinations of dust swept from the floors of hospital wards, the dispensary, or the laboratory. Once it was obtained in scrapings from the human skin. It was isolated twice from cesspools. The results of Schattenfroh and Grassberger concerning the presence of this bacillus in market milk were confirmed.

In the light of these and previous investigations *Bacillus aerogenes capsulatus* must be regarded as one of the most widely distributed bacteria.

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[1] Abstract of paper presented at the Second Annual Meeting of the American Society of Bacteriologists, Baltimore, December, 1900.

J. Bost. Soc. M. Sc., 1900-1, V, 369-370.

EYE TUBERCULOSIS AND ANTI-TUBERCULAR INOCULATION IN THE RABBIT[1]

I will take this opportunity to report the results in three rabbits which were exhibited before this Association at the last meeting in May, 1892. Each of these rabbits had been inoculated by Dr. Trudeau about two months previously, with pure culture of the tubercle bacillus into the anterior chamber of the right eye and into the right thoracic cavity. One of these rabbits had been reserved as a control; a second had been treated, after the inoculation, with injections of bacterio-protein solution; and the third with injections of the filtrate of liquid cultures of *Bacillus tuberculosis* in the manner described by Dr. Trudeau in his paper read at the last meeting, and printed in Vol. VII of the "Transactions." It will be remembered that the control rabbit showed marked tuberculous disease of the inoculated eye; the rabbit treated with bacterio-protein showed conical protrusion and opacity of the cornea, which was vascularized, and also the marks of old subcutaneous abscesses at the points of injection; and the rabbit treated with the filtrate showed clear cornea and media with small, opaque, grayish white mass in the anterior chamber and adherent to the iris. Dr. Trudeau requested me to take charge of these rabbits after the meeting, and to continue the treatment which he had begun. This treatment was carried out in accordance with the directions.

The rabbit which was treated with bacterio-protein solution died June 14, 1892. For several days previous to its death it was weak and unable to stand. It died very much emaciated and apparently from exhaustion. An abscess, developed near the right lower jaw. The following is extracted from the notes of the autopsy: The right cornea protrudes in a conical form, is opaque, and vascularized over most of its extent, and superficially ulcerated at the most prominent point. The iris is apparently represented by a thin, opaque, membranous mass adherent to the posterior surface of the cornea. The lens is dislocated into the anterior chamber. In the membranous tissue representing the iris are four or five firm, opaque, yellowish white tubercules about the size of a pin's head. The aqueous and vitreous media are turbid but not purulent. The left eye is normal.

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[1] Report of experimental study and remarks on a paper of E. L. Trudeau, before the Association of American Physicians, Washington, D. C., May 31, 1893.

Tr. Ass. Am. Physicians, Phila., 1893, VIII, 113-114.

There are old adhesions between visceral and parietal pleura of right side over a small patch where there is a small firm, opaque, whitish mass.

The scars and scabs of old abscesses and ulcers can be seen on the skin of the back at points corresponding to the injections of the bacterio-protein solution.

The abscess near the jaw contains short and long bacilli readily staining with aqueous solution of methylene blue.

There are no tubercles except the old ones in the right eye and right pleura.

The rabbit which had been treated with the filtrate of tubercle culture died October 28, 1892, in consequence of acariasis of the ears (psoroptes cuniculi) which at the time proved fatal to several of the rabbits in our stalls.

The cornea and media of the right eye were perfectly clear and normal. In the upper segment of the anterior chamber adherent to the iris was a small whitish speck, not exceeding one-half millimeter in diameter, adherent to the iris. No tubercles could be seen in the iris or in any other part of the body. The only lesions found were the customary ones of acariasis of the ears.

The control rabbit died December 19, 1892. It had been noticed to be sick and weak for several days before death. The following is from the record of the autopsy:

> The right cornea is opaque and bulging. About its centre is an ulcerated opening through which dry, opaque, cheesy material protrudes. The anterior chamber and the iris are filled with the same caseous material. The lens cannot be found. The posterior half of the eyeball is atrophied. A gland in the orbit contains several small, opaque, yellowish white tubercles, the largest one is the size of a pin's head.
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> Over the right side of the thorax is an encapsulated abscess with caseous contents 4½ cm. in length and 3½ cm. in width, lying over the pectoral and intercostal muscles. Attached to both visceral and parietal pleura of right side are eight encapsulated, pedunculated or loosely sessile, caseous masses, varying in size from a pin's head to a bean. There are no tuberculous nodules elsewhere than in the situations mentioned. The animal is emaciated and presents increased quantity of serum in the serous cavities. No bacteria can be detected in cover-slip preparations from the caseous masses.

It will be observed that none of the rabbits, not even the control, developed generalized tuberculosis. Localized tuberculosis is not uncommonly the sole result of inoculation of rabbits with tuberculous material.

The control rabbit, suffered destruction of the right eye from caseous panophthalmitis, and developed multiple caseous foci near the seat of inoculation in the right thorax.

The rabbit treated with bacterio-protein developed less extensive tuberculosis at the points of inoculation than did the control rabbit, but still it presented several small, discrete, tuberculous nodules at these points, and the cornea became bulging, opaque, and vascularized.

The rabbit treated with the filtrate showed no visible tubercles whatever in either of the two seats of inoculation or elsewhere, and died seven months after inoculation from an accidental affection, acariasis auriculorum.

The results in this series of experiments are therefore confirmatory of those already reported by Dr. Trudeau.

THE CHANGES IN THE LUNGS IN SYSTEMIC BLASTOMYCOSIS AS CONTRASTED WITH THOSE OF TUBERCULOSIS[1]

It may be of interest to recall the first observations upon this class of affections made in this country. This was in 1893, when I received from Dr. Rixford, of San Francisco, material excised from a patient with a singular affection of the skin. In this I discovered a peculiar parasite, and I subsequently handed the material over to Dr. Gilchrist, who in conjunction with Dr. Rixford published the case in full. By a remarkable coincidence, while we were engaged in studying the first case Dr. Duhring, of Philadelphia, sent to my laboratory material from the first case of blastomycetic dermatitis recognized as such. We therefore at the beginning had opportunity to compare the type of organism which causes the so-called coccidioidal disease of the San Joaquin Valley of California, and that concerned in the group of diseases which have been observed in so many instances here in Chicago and described so clearly by Dr. LeCount. We recognized that whereas the parasite in the latter multiplies in the tissues by budding, that in the California disease multiplies by endogenous sporulation. We were misled into interpreting the California parasite as a protozoön, as we did not have fresh material for cultivation, but Dr. Ophüls has since demonstrated by cultural methods that it belongs to the fungi or yeasts, and he calls it an oidium.

While not identical, both the parasites and the clinical and anatomical characters of the coccidioidal disease and of the systemic blastomycosis have much in common. The resemblance to tuberculosis is possibly even more striking in the case of the California mycosis than in the group described by Dr. LeCount, and this resemblance was fully described and emphasized in the original report of Dr. Rixford's case in the first volume of the Johns Hopkins Hospital Reports. It would have been simply impossible to have distinguished the lesions in the lungs, lymphatic glands, and other organs in this case from tuberculosis, either by the naked eye or by the microscope, without the aid of demonstration of the parasite. I know of no other disease which simulates so perfectly the characters of generalized tuberculosis.

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[1] Report of the case in which the first diagnosis of Blastomycosis was made in this country. Remarks upon a paper by E. R. LeCount, before the National Association for the Study and Prevention of Tuberculosis, Chicago, June 5, 1908.
Nat. Ass. Study & Prev. Tuberc., Tr., 1908, IV, 147-149.

The diagnosis is, of course, to be made by the microscopic demonstration of the parasite, which is not a matter of difficulty. Nor is there much difficulty in distinguishing the parasite of the so-called coccidioidal or California mycosis from that of systemic blastomycosis as observed elsewhere in this country, most frequently in Chicago. There are still open questions concerning the relationship between these two classes of disease. which have much akin.

MODE OF INFECTION AND OF DISSEMINATION OF TUBERCULOSIS IN MAN, BASED ON ANATOMICAL INVESTIGATION[1]

It is interesting to observe the contrast between pathological anatomists and bacteriologists and some clinicians, who are engaged merely in experimental work, in attempting to analyze their cases. Dr. Ravenel pointed out the inadequacy of that kind of evidence as to modes of infection. Pathological anatomists in general believe that the great preponderance of cases of pulmonary tuberculosis are referable to primary infection through the bronchi and lungs; they stand almost as a unit on that side of this disputed question. There is no question about the experimental facts as to the possibility of tubercle bacilli passing through the mucous membrane of the alimentary canal, without leaving a record behind, as the portal of entry; but, as Dr. Ophüls has pointed out, we are not justified, without further evidence, in applying these experimental results to conditions in human beings and rejecting all the older almost universally accepted ideas as to the paths of infection. This matter was threshed out very carefully in an interesting discussion in Vienna when the International Conference on Tuberculosis met there. At that time there was a most thorough and interesting presentation of the subject from all points of view, and I would recommend those who are particularly interested in this question to refer to that. One of Flügge's pupils pointed out that it takes a vastly larger number of tubercle bacilli to produce infection through the alimentary canal than it does through the air passages. He believes that a single tubercle bacillus is sufficient to produce infection through the air passages; from what Dr. Webb has taught us it is questionable whether a single tubercle bacillus, or less than a hundred tubercle bacilli, would be sufficient to produce infection by inhalation; whereas, it takes millions of times more tubercle bacilli to produce infection through the alimentary canal than it does from the respiratory passages. This is a significant fact.

Dr. Ophüls has expressly excluded from his consideration conditions in children. These are the ones where the widest observations have been made. There is no doubt that the great majority of cases of tuberculosis in children show lesions, and sometimes evidence of old lesions, in the peribronchial lymphatic glands. It has been shown by Hamburger, who has made very careful examinations in these cases of pulmonary lesions, that they do not usually go on to the formation of pulmonary ulcerative phthisis; but that the lesions in the peribronchial glands in children are small, with evidence of their being really old lesions in the lungs. They are not necessarily apical lesions. With tuberculous infection in children the lesions are probably in the lungs, and almost, at the same time, in the peribronchial lymphatic glands; and with it there is a remarkable tendency to the extension of the disease to other lymphatic glands. It is difficult to reconcile oneself with the idea that that kind of localization of the lesions in the peribronchial lymphatic glands can be explained by infection through the alimentary canal. So I believe in the older view that the infection is in the majority of instances, in cases of pulmonary tuberculosis, both in infants and adults, through the respiratory tract, and not through the alimentary canal; and, at the same time, I admit that we had no adequate conception in the old days as to the importance of the alimentary route. Admitting all of that, it still remains that the majority of cases are due to infection through the respiratory route, and, in that respect, I have to differ from my friend, Dr. Ravenel, who, as is well known, is a strong advocate of the other view, that the infection is usually through the alimentary canal, his view being based upon experimental work.

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[1] Remarks on paper by William Ophüls before the National Association for the Study and Prevention of Tuberculosis, Denver, Col., June 20-21, 1911.
Nat. Ass. Study & Prev. Tuberc., Tr., Phila., 1911, VII, 409-411.

We have had this subject presented by an expert pathological anatomist, who insists upon the fact that pathological anatomists have a word to say on this subject as to the routes of infection.

WHAT SHALL BE THE MODE OF PROCEDURE IN DETERMINING THE PATHOGENESIS OF BACTERIA FOUND IN WATER?[1]

Of the diseases which are known to be conveyed by contaminated drinking water the most important are Asiatic cholera and typhoid fever. There is evidence that gastric and diarrhoeal affections may also be caused by drinking waters, but we have no positive knowledge of the microorganisms which may be here concerned. Although *Amoeba dysenteriae* has not been demonstrated outside of the body, there is strong evidence that amoebic dysentery, and probable evidence that other forms of dysentery, may be caused by contaminated drinking water. There are some writers who strongly advocate the view that malaria may be conveyed by the drinking water, but positive proof of this view has not been furnished. The possibility of the conveyance of scarlet fever and diphtheria by water cannot be denied, but there is not much evidence in support of this opinion. Pyogenic bacteria, and various bacteria pathogenic to the lower animals, have been found in water.

Outside of cholera-infected localities typhoid fever is the disease most frequently suspected of conveyance through the agency of drinking water, and therefore the water problem oftenest presented to the bacteriologist is to determine whether or not the water in question contains, or is likely to contain, the typhoid bacillus.

By the work of Schottelius, Gruber, and Koch we now possess methods which offer a good prospect of isolating from water the cholera spirillum, should this organism be present, although this isolation has been rendered more difficult by the discovery of a large number of water vibrios which bear more or less resemblance to the cholera vibrio. The studies of R. Pfeiffer show that a most important differential criterion of the cholera spirillum is furnished by immunization of animals with this organism, so that we must recognize the animal test as an important factor in the identification of this microorganism.

We possess no equally satisfactory method for the determination of the presence of the typhoid bacillus in water. While we cannot say that the isolation of this bacillus from water by our present methods is altogether hopeless, nevertheless the difficulties are so great that there is a justfiable skepticism whether any of the reported isolations of this bacillus from drinking water rest upon the positive identification of the bacillus. With our present methods the most which can be expected from the biological examination of water as regards this question is the determination, not of the actual presence of the typhoid bacillus, but of the possibility or probability of its presence.

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[1] An address before the Convention of Bacteriologists, under the auspices of the Committee on the Pollution of Water Supplies of the American Public Health Association, New York City, June 21, 1895.

Am. Pub. Health Ass., Rep., Concord, N. H., 1895, XX, 502-516.

So far as this method of examining water is concerned, our principal guide at present in drawing conclusions as to the possible presence of the typhoid bacillus in suspected drinking water is the recognition of foecal bacteria, and more particularly of members of the colon group of bacteria, which are the chief and most characteristic representatives of intestinal bacteria. By the work of Vincent, Peré, Wurtz, Mathews, T. Smith, Burri, and others we possess methods which enable us to isolate with much certainty from water the colon bacillus. For this purpose it is possible to submit to examination a litre or more of water. These improvements in technique have shown that the colon bacillus is a wide spread organism in water, and in small number may be present even in the purest waters. The determination of the mere presence of this bacillus in water does not, therefore, suffice for the condemnation of the water. Attention must be paid to the number of colon bacilli present, and the determination of this point is not always easy. Moreover, where the decision rests upon quantitative rather than qualitative determination of bacteria, there must necessarily be opportunities for considerable differences of opinion even among experts.

Colon bacilli are distributed in external nature no less from the excrements of animals than from those of human beings, and although water seriously polluted with animal excrements is not fit for drinking, nevertheless we have no reason to suppose that typhoid bacilli are derived from animal excrements.

It is evident that the biological examination of water does not by itself alone at present suffice to solve the important sanitary problem here under consideration, although it affords valuable information. We must resort also to all the other methods at our disposal for the sanitary examination of sources of supply of drinking water, and not the least of these is the careful inspection for channels of contamination. Although these sources of contamination may be manifest to casual and untrained inspection, in general this inspection should be considered as no less a matter for expert study than the biological or the chemical examination of water.

Only one point regarding the identification of suspected typhoid and colon bacilli found in the water properly belongs to my theme, and that is, whether the inoculation of animals affords us any help in this identification. These bacilli, as is well known, may manifest interesting pathogenic properties when inoculated into animals, but these are not so constant or of such character as to enable us to rely upon them as valuable differential factors. Essentially similar results may be obtained by the inoculation of animals with virulent colon bacilli as with typhoid bacilli, as has been shown by the experiments of Blachstein and myself.

The question whether the immunization of animals with the colon bacillus, or with the typhoid bacillus, may assist us in the identification of these bacilli, and more particularly in the identification of the typhoid bacillus in the same way as it helps in the differentiation of the cholera spirillum, does not seem to me to have been as yet satisfactorily answered, notwithstanding a certain amount of work upon it. The results have been conflicting, but it is not improbable that we may find in this procedure a valuable differential criterion for the typhoid bacillus. The procedure would be, on the one hand, the immunization of rabbits or guinea pigs or mice with culture products of an undoubted typhoid bacillus, and the subsequent determination of the presence or absence of immunity from the suspected typhoid bacillus; and, on the other hand, the immunization of the animals with cultures or products of the suspected bacillus and their subsequent inoculation with undoubted virulent typhoid cultures or their products. A number of preliminary problems must, however, be solved before we can determine the value of this immunizing test.

Although the cholera spirillum and the typhoid bacillus claim the first place among the pathogenic bacteria which may be found in water, these are not all of the disease-producing bacteria which may be present. Mention has already been made of the occasional presence of pyogenic staphylococci and streptococci. *Bacillus proteus* and *Bacillus pyocyaneous* are not without some pathogenic significance, and they have been detected in water. Of the bacteria pathogenic to animals there are several which must find opportunity to gain access to water. Mention may be made especially of the anthrax bacillus, the hog cholera, and the chicken cholera bacillus. As is well known, a number of pathogenic bacteria, chiefly of interest to us in our laboratory experiments, have been isolated from water, usually, however, from water which no one would think of drinking.

Although pathogenic bacteria in general do not multiply in drinking water, and frequently quickly die out, still they may survive in certain drinking waters a long time. The opinion that cholera and typhoid bacteria cannot survive in drinking water more than a few days has been shown to be incorrect. They may live a month, or even longer, in some unsterilized drinking waters.

Our search for pathogenic bacteria in water is much facilitated by the fact that bacteria pathogenic for warm blooded animals, if capable of development in our artificial cultures, will grow at the temperature of the incubator. By cultivating at 36° or 37° C. we are enabled to eliminate a large number of the ordinary water saprophytes. There are, however, bacteria pathogenic for frogs and other cold-blooded animals which do not grow at these high temperatures.

In attempting the isolation of particular species of pathogenic bacteria we can sometimes use to advantage elective cultures media, a procedure which is likely to be improved and still further elaborated by subsequent research.

In testing as to the presence of pathogenic or toxic germs in water, it seems to me to require no argument at the present day and before this convention that such tests should be made with the pure cultures of the isolated bacteria, or if for any reason it is desired to determine the effects of inoculation of mixed cultures, this should be done with precise knowledge of the bacteria concerned, a knowledge which can be gained only by the isolation of pure culture of the individual species. The adoption of such procedures as the inoculation of animals with impure bouillon cultures obtained simply by the addition of water to bouillon is contrary to the accepted canons of modern bacteriology and does not afford information which is clear in its interpretation.

I should like to see the test of pathogenesis adopted as a routine procedure in the determination of the characters of all species of bacteria, and I would even go so far as to suggest the inoculation of frogs or other cold-blooded animals in the case of bacteria which grow only at ordinary temperatures. Our blank forms for inserting the descriptions of bacterial species contain the word "pathogenesis" as one heading, and the space belonging to this caption should not be left blank when we record the differential characters of a species.

It is true that virulence is one of the most variable of the characters of bacteria, and that cultures of pathogenic species isolated from external sources are particularly prone to be of little or no virulence. These facts, however, do not justify us in dispensing with the test of virulence in our studies of bacteria from the water and other sources. The determination of loss of virulence under various conditions is in itself an interesting and important fact.

In testing bacteria isolated from the water for pathogenic properties I do not know that there are any technical points concerned different from those applicable to bacteria from other sources. Inasmuch as it is the purpose of this convention to establish so far as practicable certain standards of bacteriological procedure, especially in the line of greater precision, it will be appropriate to speak of certain points in the technique of testing for pathogenic properties.

It is important to distinguish between actual infection and intoxication resulting from the inoculation of bacteria, although this distinction is by no means always easy, as it is apparent from the controversies as to whether inoculation with the typhoid bacillus, the colon bacillus, or the cholera spirillum causes death by infection or by pure intoxication without multiplication of the inoculated bacteria. These controversies have been settled in favor of the view, that according to varying conditions each of these species may produce either infection or simple intoxication. Where large doses of cultures are required to produce symptoms or to cause death, there should be suspicion of pure intoxication, and especial attention should be given to the distribution of the bacteria in the animal body and to evidences of multiplication of the bacteria in the living animal. It is of course understood that even in infections bacteria do injury through their toxic products.

We are too much in the habit of considering the death of the animal following inoculation as the sole test of pathogenesis. Animals, as well as human beings, may recover from disease, even severe forms of disease, and it is well to examine carefully for evidences of pathogenic effects which may not prove fatal. The use of the clinical thermometer is an aid in this examination, but the observer should be familiar with the limits of physiological variation in the temperature of the animal concerned and of various causes, such as excitement, struggling, immobilization of the animal, which influence the temperature. Cultures can be made from the blood and other parts of the living animal. I have been much interested in the study of a nonfatal peritonitis in the mouse produced by a pseudotuberculosis bacillus. It was possible by inserting the needle of a sterilized hypodermic syringe into the scrotum to withdraw exudate from the peritoneal cavity and to examine this bacteriologically and microscopically. Notwithstanding the production of so large an amount of exudate as to greatly distend the abdomen and scrotum, and notwithstanding the demonstration of very great multiplication of the specific bacillus in the peritoneal cavity the animal recovered completely. I have also had opportunity to study a similar form of nonfatal pleurisy in the animal.

The general rule, as regards the selection of cultures to be tested for virulence, is to use either bouillon or agar cultures which have developed for forty-eight hours in the thermostat. Dosage can be more accurately expressed when bouillon cultures are used, and in general these are to be preferred. The use of forty-eight hour bouillon cultures has been the recognized rule in testing the diphtheria bacillus for its virulence. Nevertheless I have found with this bacillus, as well as with others, that inoculation with forty-eight hour agar cultures may give positive results when the bouillon cultures fail. It is sometimes useful, especially when the growth in bouillon cultures is not abundant, to reinforce the bouillon cultures with the growth on an agar tube at the time of inoculation. The solid growth on an agar culture can be inserted into the animal with the platinum loop, or a suspension of this growth can be made in a small quantity of physiological salt solution or bouillon and the inoculation made with a syringe. The turbid fluid at the bottom of an agar culture, perhaps reinforced by mixture with some of the solid growth, is often good material to select for inoculation with a syringe or platinum loop. There are of course circumstances where it is desirable to use fresher or older cultures than those specified. When it is desired to determine the presence of toxic substances in the cultures, of course older cultures will be used, the general rule being the selection of bouillon cultures three weeks old grown in the thermostat. When the bacteria are to be eliminated, the filtrate from a Pasteur or similar filter will be used.

Where fluid cultures are used dosage can be most accurately expressed in percentage of the weight of the animal. In all cases the weight of rabbits and guinea pigs used for the experiment should be stated. In general young animals are more susceptible than old animals to even proportionately the same doses of bacteria. The dosage of cultures on solid media cannot be so accurately expressed as that from liquid cultures. By the use of the platinum loop, fairly satisfactory results as regards dosage can be obtained in the hands of one and the same observer, as has been shown by the studies on this point of R. Pfeiffer in inoculations of agar cultures of the cholera spirillum. Where liquid cultures or suspensions of solid cultures are used a fairly accurate idea of the number of bacteria inoculated can be obtained by counting the bacteria in a known quantity of fluid. Nuttall's method gives particularly precise results on this point. This somewhat laborious procedure will not, however, be likely to be adopted except for certain special purposes. Plate cultures also can be used to determine the number of living bacteria present in a known quantity of the fluid used for injection.

It should be noted at the time of inoculation whether any of the material inoculated escapes from the wound, and, so far as possible, allowance should be made for this.

No rules of general application can be laid down as regards the amount of culture to be injected. Perhaps from one-half to one per cent of the body weight in the case of fluid cultures, and one or two good sized platinum loops from solid cultures, will represent the usual doses with which the first test of the possible virulence of a culture will be made. Positive results with these large doses should be followed by the inoculation of smaller quantities.

It is desirable to test the possible virulence of the cultures upon each of the three species of animals—mice, guinea pigs, and rabbits—ordinarily used for this purpose in our laboratories. These animals differ in susceptibility to different species of pathogenic bacteria. If only one animal is used, it is best to select the mouse, as being in general, although by no means always, the most susceptible. Either white mice or the ordinary house mouse may be used. I have not observed any special differences of susceptibility between these two varieties.

The simplest and most common procedure is subcutaneous inoculation, but inoculation into the peritoneal cavity or directly into circulation, is often (not invariably) a more severe test. For the technique of injection into the ear-veins of rabbits, I would refer especially to the description of this procedure given by Abbott in his " Principles of Bacteriology."

Where a single test of the virulence of a culture is to be made, the most severe is in general direct injection of the culture into the peritoneal cavity of a mouse. When this fails after a good dose (say 0.1 to 0.3 c. c.), it is not likely that other methods will be successful in causing the death of the animal, although it may be interesting to observe the local effects of subcutaneous or cutaneous inoculation.

The inoculated animal should be kept under observation weeks and sometimes even months after it has appeared to survive the effects of the inoculation. Experience with the hog-cholera bacillus, the typhoid bacillus, the colon bacillus, and even with the lanceolate micrococcus, has shown that death may occur so late that the animal may have been dismissed from further observation. Inoculated animals, if put back into the cage with others, may be marked in some way to distinguish them. In general it is best not to use animals which have survived inoculation for further tests of pathogenesis of bacteria. We cannot control, without special study, the influence which a previous inoculation may have had upon the susceptibility of the animal to the same specie or to other species of bacteria.

As regards the choice of syringe to be used in inoculating animals, this is a matter which will be determined largely by personal choice, based upon experience with one or another variety. My own preference is for the ordinary hypodermic syringe. It can be readily obtained, and cheap forms can be used. I find no difficulty in sterilizing these syringes by filling them with 5 per cent carbolic acid, allowing them to remain thus filled for half an hour or more and then washing them out thoroughly five or six times with sterilized water, salt solution, bouillon, or other sterile indifferent fluid. When filled with carbolic acid the barrel of the syringe fits loosely in the mouth of a bottle filled with carbolic acid, into which the needle dips.

The platinum needle or loop used for subcutaneous inoculations should be of tolerably thick wire, so that the needle is stiff enough to push into the subcutaneous tissue without bending.

I see no object in attempting to sterilize the skin over the seat of inoculation. Such attempts are futile, unless an eschar is produced by thorough burning. It suffices simply to cut the hair close over the site of inoculation. An incision is then to be made with sterilized scissors or scalpel into the subcutaneous tissue. The beginner is likely to fail to cut as deeply as the subcutaneous tissue, and encounters a great obstacle in attempting to force the needle into the dense cutis.

Upon the death of the animal the seat of inoculation, if subcutaneous, should first be exposed and carefully examined bacteriologically and microscopically.

In making the post mortem examination it seems to me unnecessary to resort to any elaborate antiseptic precautions in exposing the different viscera, unless especial attention is to be paid to cultures from serous cavities. The skin need not be, in fact cannot be readily, sterilized, and it suffices to lay the hairs by simple wetting with water over and around the line of incision through the skin. This incision need not necessarily be made with sterilized instruments, or the scalpel and scissors may be slightly flamed. It requires far less heating in the flame of a Bunsen burner than is generally employed to sterilize metal instruments. The heat need not be so great as to seriously damage the steel. Incisions through the skin approximately at right angles to the long median incision and extending along the extremities very much facilitate the disposition of the flaps, which can thus be kept out of the way of the subsequent examination, without resorting to pins or other devices.

Cultures should be made at once after opening the animal. It is important to determine the distribution of the bacteria in the blood and various organs, and as a matter of routine procedure it is well to make cultures from the peritoneal cavity, spleen, liver, kidneys, lungs, and heart's blood. Especial care will of course be taken to obtain cultures from any apparent local focus of disease. My custom is, after opening the peritoneal cavity and before removal of the sternum, to make cultures first from the peritoneal cavity, then from the spleen, the liver, and the kidneys. The sternum is then removed and cultures made, if need be, from the pleural cavity, then from the lungs and the heart's blood. I consider it important to burn thoroughly the surface of each viscus with a red hot scalpel or other metal, at the site where the needle is thrust in to obtain the material for inoculation. This precaution renders unnecessary the elaborate preliminary steps to secure a sterile field, which have sometimes been recommended. It destroys microorganisms which may have entered accidentally from the air, or by contact, and it eliminates from the culture the bacteria which may simply be present on the serous covering of the organs, and not present in the substance of the organ. As by this thorough sterilization of the surface bacteria may be killed to a certain depth, the platinum needle should be thrust well into the substance of the organ, and this is facilitated if a small incision is first made with a hot scalpel through the scorched area on the surface.

The amount of material to be used for inoculating the culture tubes will vary with the number of bacteria likely to be present in the material. These may be so abundant that the insertion of a straight needle brings away enough, or a loop may be used to secure a larger quantity, or it may be necessary to remove a bit of tissue with a sterilized instrument. Especially constructed platinum scoops or spears and other devices may be left to the ingenuity of each experimenter, where large amounts of material are to be secured, or other purposes, such as ease of penetration into a dense organ, are to be subserved by their use.

When it is desired simply to recover in pure culture from the organs of the inoculated animal the species of bacterium inoculated, simple stab, line, or smear cultures will suffice, but the correct procedure for determining approximately the number of bacteria in different situations, and for insuring, according to Koch's principles, pure cultures from single colonies, is, of course, to make plate or roll cultures. As a rule these will be agar cultures in Petri's dishes.

After the culture tubes have been inoculated and labelled, the microscopical examination by cover-glass preparations is to be made of the blood and other fluids and of the organs. This should never be neglected. It affords information as to the number and distribution of the bacteria, their arrangement, the presence or absence of capsules, their morphology in the animal body, and their staining reactions in this situation.

Sections can at once be made of the organs with the freezing microtome, and these are the most useful in determinig the existence of fatty and other degenerations, which often escape observation in the study of sections of the hardened tissues. These sections can be used also for staining the bacteria in the tissues, but not much is to be gained by this, as better results are obtained in staining the bacteria in the hardened organs.

Alcohol is the most useful hardening agent. The meagre and unsatisfactory descriptions of the histological lesions furnished by bacteriologists in general is much to be regretted, but this perhaps, cannot always be otherwise, as bacteriologists are not always pathologists. Very considerable interest attaches to the pathological study of our experimental infections, and I would suggest that especial attention be paid to the careful histological examination of the organs and tissues in our experimental infections. Matters of interest in themselves, and sometimes of diagnostic value in determining species, will thus be brought to light.

If the culture proves to be of only slight virulence, the attempt may be made to increase the virulence by successive passages through the animal body. Sometimes this is best accomplished by inoculation directly from animal to animal, and sometimes by inoculation of fresh cultures from the animal body.

* * * * * * * * * * * *

It is generally agreed that the dosage of fluid inoculations is best expressed in percentages of the weight of the animal; but we are also to remember that animals of different ages and weight vary in susceptibility. A large animal is more resistant to the dose, in proportion to its weight. The small animal is simply more susceptible. The question in my mind is whether it is desirable to select animals of weight within certain limits, not only for diphtheria, but for other things.

* * * * * * * * * * * *

In regard to the thermometer, Mr. Chairman, I agree with you that it is worth while to take the temperature in the rectum. My experience has been almost entirely with rabbits. The temperature of the rabbit varies within a very wide limit,—from 101° to 104° F. It makes a great difference, also, whether the animal struggles or not in taking the temperature. It is well known that a rabbit's temperature falls when it is fastened down for any length of time, and it is also desirable to have a record of the normal run of the animal's temperature.

The importance of keeping inoculated animals a long time under observation is illustrated by our experiment with the inoculation of rabbits with the colon bacillus, and the typhoid bacillus. After the experiment was supposed to be over they were marked with various colors to identify them. I found that they died sometimes as long as four or five months after inoculation. The colon bacillus and the typhoid bacillus are able to stay almost indefinitely in the bile of the animal, and may produce eventually the death of the animal, in one instance nearly a year after inoculation. This is an extreme case. The longest period is usually four months. The hog-cholera bacillus sometimes will cause the death of the animal after a month or six weeks, particularly of the mouse. In all cases animals should be marked so as to identify them in the future.

As regards the syringe, I think that is a point which every one makes up his mind about from experience.

ERRATA

L. F. T. means line from top; L. F. B. means line from bottom

Page	Location of Error		Correction
26	L. F. T. 3	“though”	should read through
65	L. F. T. 11	“also”	should be omitted
69	L. F. B. 18	“arbin”	should read abrin
78	L. F. T. 3	“ration”	should read ratio
80	L. F. B. 6	“for”	should read of
101	L. F. B. 3	“activity”	should read actively
146	L. F. T. 9	“capsulates”	should read capsulatus
158	L. F. B. 2, 6, 8, 10 and other places in volume II, “empyaema” should read empyema		
163	L. F. T. 13	“difinite”	should read definite
202	L. F. B. 2	“are”	should read is
229	L. F. T. 12	“Carnegia”	should read Carnegie
229	L. F. T. 20	“if”	should read of
255	L. F. B. 3	“pseudodiphthritic”	should read pseudodiphtheritic
261	L. F. B. 10	“ar”	should read as

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